



12-1-1974

The Distribution of Histone Polypeptides and It's Methylated Basic Amino Acids from the Various Organs of the Rat

Subhas Chakrabarty

Follow this and additional works at: <https://commons.und.edu/theses>

Recommended Citation

Chakrabarty, Subhas, "The Distribution of Histone Polypeptides and It's Methylated Basic Amino Acids from the Various Organs of the Rat" (1974). *Theses and Dissertations*. 468.
<https://commons.und.edu/theses/468>

This Thesis is brought to you for free and open access by the Theses, Dissertations, and Senior Projects at UND Scholarly Commons. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of UND Scholarly Commons. For more information, please contact zeinebyousif@library.und.edu.

THE DISTRIBUTION OF HISTONE POLYPEPTIDE
AND IT'S METHYLATED BASIC AMINO ACIDS
FROM THE VARIOUS ORGANS OF THE RAT

by
Subhas Chakrabarty

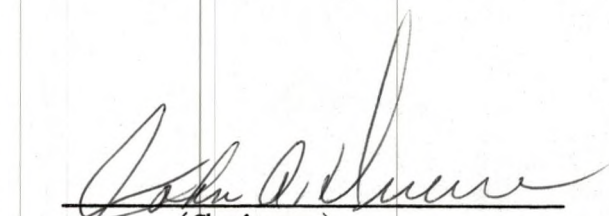
Bachelor of Science, University of North Dakota 1973

A Thesis
Submitted to the Faculty
of the
University of North Dakota
in partial fulfillment of the requirements
for the degree of
Master of Science

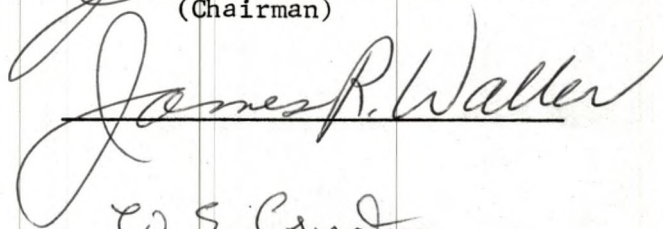
Grand Forks, North Dakota

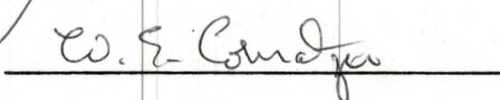
December
1974

This thesis submitted by Subhas Chakrabarty in partial fulfillment of the requirements for the Degree of Master of Science from the University of North Dakota is hereby approved by the Faculty Advisory Committee under whom the work has been done.



(Chairman)





Dean of the Graduate School

Permission

Title THE DISTRIBUTION OF HISTONE POLYPEPTIDE AND IT'S
METHYLATED BASIC AMINO ACIDS FROM THE VARIOUS ORGANS
OF THE RAT

Department Microbiology

Degree Master of Science

In presenting this thesis in partial fulfillment of the requirements for a graduate degree from the University of North Dakota, I agree that the Library of this University shall make it freely available for inspection. I further agree that permission for extensive copying for scholarly purposes may be granted by the professor who supervised my thesis work or, in his absence, by the Chairman of the Department or the Dean of the Graduate School. It is understood that any copying or publication or other use of this thesis or part thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of North Dakota in any scholarly use which may be made of any material in my thesis.

Signature Subhas Chakrabarty

Date Dec., 1974

ACKNOWLEDGEMENTS

The author wishes to express sincere gratitude to Dr. John A. Duerre for his helpful suggestions and expert guidance without which this work could not have been completed.

The author also expresses gratitude to Dr. James R. Waller and Dr. William E. Cornatzer for their constructive criticism.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	iv
LIST OF TABLES.....	vi
LIST OF ILLUSTRATIONS.....	vii
ABSTRACT.....	ix
INTRODUCTION.....	1
HISTORICAL REVIEW.....	3
MATERIALS AND METHODS.....	10
Preparation of Labelled Nuclei.....	10
Preparation of Chromatin.....	10
Preparation of Histones.....	11
Purification of Histones by Gel Filtration.....	13
DEAE-Cellulose Chromatography.....	13
Polyacrylamide Gel Electrophoresis.....	14
Basic Amino Acid Analysis.....	15
Determination of the Relative Distribution of Histone Components.....	21
RESULTS.....	22
DISCUSSION.....	65
SUMMARY.....	69
APPENDIX.....	72
LITERATURE CITED.....	75

LIST OF TABLES

Table	Page
1. The Distribution of Histone Fractions from the Various Organs of the Rat.....	25
2. The Distribution of Basic Amino Acids in the F _{2a1} Histone from Rat Cerebellum.....	58
3. The Distribution of Basic Amino Acids in the F _{2a1} Histone from Rat Cerebrum.....	59
4. The Distribution of Basic Amino Acids in the F _{2a1} Histone from Rat Liver.....	60
5. The Distribution of Basic Amino Acids in the F _{2a1} Histone from Rat Thymus.....	61
6. The Distribution of Basic Amino Acids in the F _{2a1} Histone from Rat Kidney.....	62
7. The Distribution of ϵ -N-Monomethyllysine and ϵ -N-Dimethyllysine in the F _{2a1} Histone from the Various Organs of the Rat.....	63

LIST OF ILLUSTRATIONS

Figure	Page
1. Elution Profile of a Standard Amino Acid Sample.....	17
2. Reference Curve for the Determination of Protein...	20
3. Polyacrylamide Gel Electrophoresis of Total Histones Extracted from Rat Cerebellum, Cerebrum, Liver, Thymus and Kidney.....	24
4. Elution Profile of Rat Cerebellum Histones on Bio-Gel P-10.....	27
5. Elution Profile of Rat Cerebrum Histones on Bio-Gel P-10.....	29
6. Elution Profile of Rat Liver Histones on Bio-Gel P-10.....	31
7. Elution Profile of Rat Thymus Histones on Bio-Gel P-10.....	33
8. Elution Profile of Rat Kidney Histones on Bio-Gel P-10.....	35
9. Polyacrylamide Gel Electrophoresis of the Five Histone Components from Cerebellum.....	38
10. Polyacrylamide Gel Electrophoresis of the Five Histone Components from Cerebrum.....	40
11. Polyacrylamide Gel Electrophoresis of the Five Histone Components from Liver.....	42
12. Polyacrylamide Gel Electrophoresis of the Five Histone Components from Thymus.....	44
13. Polyacrylamide Gel Electrophoresis of the Five Histone Components from Kidney.....	46
14. Fractionation of the Basic Amino Acids from Rat Thymus F ₁ Histone on Beckman PA-35 Resin.....	48

Figure		Page
15.	Fractionation of the Basic Amino Acids from Rat Thymus F _{2a2} Histone on Beckman PA-35 Resin.....	50
16.	Fractionation of the Basic Amino Acids from Rat Thymus F _{2b} Histone on Beckman PA-35 Resin.....	52
17.	Fractionation of the Basic Amino Acids from Rat Thymus F _{2a1} Histone on Beckman PA-35 Resin.....	54
18.	Fractionation of the Basic Amino Acids from Rat Thymus F ₃ Histone on Beckman PA-35 Resin.....	56

The Distribution of Histone Polypeptide and It's Methylated
Basic Amino Acids from the Various Organs of the Rat

Subhas Chakrabarty, M.S.

The University of North Dakota, 1974

Faculty Advisor: Professor John A. Duerre

Total histones were extracted from the cerebellum, cerebrum, liver, thymus and kidney of thirty 12 day old albino rats. Polyacrylamide gel electrophoresis of twenty-five μ g of histone from the various organs was carried out. The F_1 , F_3 , F_{2a2} plus F_{2b} and F_{2a1} components were separated into distinct bands and the relative per cent of the various bands on the gels were determined by scanning the gels through a densitometer. The relative distribution of the histone components from the various organs was compared. It was found that there was no significant difference in the distribution of the histone components from the organs studied. The distribution of the various histone components does not exhibit any form of tissue heterogeneity.

The five histone components (F_1 , F_{2a2} , F_{2b} , and F_{2a1}) from the nuclei of the cerebellum, cerebrum, liver, thymus and kidney of forty-six 15 day old albino rats, which had previously received 10 μ curies of L- $[^3H]$ lysine and 5 μ curies of L- $[^{14}C]$ -methylmethionine, were extracted. The animals were sacrificed 14 days after the injection of the isotopic compounds. The various histone components

from the various organs were further purified by gel filtration and their purity checked by polyacrylamide gel electrophoresis. The purified histone components were hydrolyzed in 6 N HCl in vacuo and the basic amino acids fractionated on Beckman P-35 resin. Quantitation of the basic amino acids were carried out with the aid of an automatic amino acid analyzer. It was found that only the F_{2a1} and F_3 histones were methylated significantly. The products of methylation in the F_{2a1} histones were ϵ -N-monomethyllysine and ϵ -N-dimethyllysine, with a predominance of ϵ -N-dimethyllysine. The products of methylation in the F_3 histones were ϵ -N-monomethyllysine, ϵ -N-dimethyllysine and ϵ -N-trimethyllysine. No methylarginine was detected in any of the histones analyzed. The amount of methyllysine in the F_{2a1} histones varied from 0.67 moles/mole in kidney to 0.93 moles/mole in cerebrum. Liver F_{2a1} had the second highest amount of methyllysine next to cerebrum. The slightly higher methyllysine content in the cerebrum and liver may reflect the maturity of those cells in these particular organs. Since methylation of the lysyl residues occurs after the synthesis of the polypeptide chain, methylation of the histones in newly formed cells might be slightly less in older, matured cells. Pure F_3 histones were obtained by repeated gel filtration after which quantities available did not allow for complete analysis. It appears that methylation of the lysyl residues in the histone polypeptides does not contribute to tissue heterogeneity.

INTRODUCTION

Histones are basic, small molecular weight proteins that are found associated with DNA in the nuclei of all eukaryotic cells. There are five major types of histone polypeptides that occur in fairly constant amounts and uniform proportions in the chromatin of different eukaryotic cell types and they are ubiquitous among differentiated organisms. The biological roles of the histones are not fully understood. These proteins have been greatly conserved over the course of evolution. Much structural similarity exists among the histones from a wide variety of species. Histone fraction F_{2a1} from calf thymus has an amino acid sequence which differs from the corresponding histone in pea seedling only in the substitution of a lysine for an arginine and a valine for an isoleucine (1). This striking conservation suggests that these proteins must be fulfilling a crucially important biological role.

Many investigators feel that the biological roles of the histones are multiple. They complex to DNA and cause such DNA to be inactive as a template for RNA polymerase. Histones play a role in the supercoiling of DNA. A particular histone even forms interpolypeptide disulfide bridges during metaphase, apparently stabilizing the chromosomes in a highly condensed state (2). In addition, histone polypeptides are subjected to chemical modification after synthesis in vivo, such as the methylation of the ϵ -N-amino group of lysine.

The exact role of methylation of the histone polypeptide chain is not yet understood. However, it has been suggested that the modification of the histone polypeptide has an effect on the DNA-histone interaction and that it may be involved in regulating gene expression by affecting the transcription process (3). Methylation of histones appears to be a very specific phenomenon occurring after histone synthesis is complete (4). Some investigators feel that chemical modification of these histone polypeptides could contribute to tissue heterogeneity.

It is the purpose of this investigation to determine the distribution of methylated basic amino acids in the various histone components from different organs of the rat. The histones from the cerebellum, cerebrum, liver, thymus and kidney will be compared. In addition, the distribution (relative percent) of the various histone components from the above organs will also be compared.

HISTORICAL REVIEW

The presence of ϵ -N-methyllysine was first identified in the flagellar protein of Salmonella typhimurium by Amber and Rees in 1959 (5). Keireidge in 1963 demonstrated that methionine was the methyl donor for ϵ -N-methyllysine in flagellin (6). The presence of ϵ -N-methyllysine in histone was first detected by Murray (7). He isolated an unidentified component from histone hydrolysates. The compound was identified as ϵ -N-methyllysine by comparing its behavior on ion-exchange chromatography and paper chromatography with that of hydrolyzed flagellin, and from the mass spectrum of its ethyl ester. This amino acid now has been obtained from histone of many sources, such as calf kidney, calf liver, calf spleen, rabbit liver, rabbit kidney, rabbit spleen, lamb liver and rat liver. Murray (7) was able to isolate radioactive ϵ -N-methyllysine from histone prepared from various tissues of rabbits which had been injected previously with L- $[^{14}\text{C}\text{-methyl}]$ -methionine. He concluded that methionine was the methyl donor involved in the formation of ϵ -N-methyllysine.

Allfrey et al (8) presented evidence showing methylation of histones very probably occurred after the completion of the polypeptide chain synthesis. Concentrations of puromycin which inhibited the synthesis of non-histone proteins by over 90 per cent, and which lowered $[^{14}\text{C}\text{-lysine}]$ uptake into the histones by 73 per cent, had no

effect on methylation of the histones in isolated calf thymus nuclei. They further showed that the peak in methylation occurred at a time when the rates of histone synthesis and DNA synthesis had already begun to decline and that methylation did not correlate with histone, non-histone protein, nor DNA synthesis (9). In studying the methylation and turn over of rat brain histones in vivo, Duerre and Lee (10) concluded that the methylation of all lysyl residues in the different brain histones appeared to be a specific event occurring at the time or shortly after the synthesis of the polypeptide chain. They also presented evidence (11) to show that some of the specificity for methylation resided within the arrangement of the histones on DNA, that the specificity for methylation of the lysyl residues in the polypeptide chain was lost when histones were dissociated from DNA and that methylation occurred after the histones were bound to DNA.

It is now known that the occurrence of ϵ -N-methyllysine in the basic proteins of the nucleus is not due to an incorporation of methylated amino acid itself, but represents instead a transfer of methyl groups from methyl donors to the free ϵ -amino groups of lysyl residues in the polypeptide chain. Paik and Kim (12) in 1965 were able to identify in calf thymus nuclei an enzyme which catalyzed the methylation of the ϵ -amino group of lysine. The methyl groups were donated by S-adenosyl-L- ^{14}C -methyl-methionine. Alvin and Dalia reported that the 150,000 x g supernatant fraction of rat organs was capable of transferring methyl groups of S-adenosyl-L- ^{14}C -methyl-

methionine to lysyl residues of histones and some non-histone proteins (13). Paik and Kim in 1970 were able to solubilize and partially purify an enzyme from calf thymus nuclei which methylates the ϵ -amino group of lysyl residues in histones (14). They also found this enzyme in rat spleen, lung, testis, liver, kidney, brain, heart and muscle, with the highest enzymic activity in the thymus, spleen and lung (14). This enzyme was found to be most active towards various histones, and especially arginine-rich histone.

The enzyme responsible for the methylation was localized in the nucleus, and enzymic activity was elevated whenever cell proliferation was accelerated such as that of fetal brain (10) or growing liver (9). Therefore, a question arises as to the relative turnover rate of the methyl groups in comparison to the polypeptide chains. Paik et al reported approximately 2% of the incorporated methyl groups were turning over in HeLa S-3 cell culture (15). Paik and Kim also reported the presence of a demethylase in rat kidney (16). However, studies on the in vivo methylation and turnover of rat brain histones by Duerre and Lee (10) indicated that the ϵ -N-methyl groups on the lysyl residues of brain histones do not turn over independently from the backbone of the polypeptide chain.

Paik and Kim reported in 1967 that the hydrolysates of calf thymus arginine-rich histone contain ϵ -N-dimethyllysine as well as ϵ -N-monomethyllysine (17). They also showed that ϵ -N-dimethyllysine is a natural component of histones. ϵ -N-dimethyllysine had not been detected previously because it eluted from the amino acid

analyzer column together with the monomethyl derivative under the conditions recommended by Murray (7). When isolated calf thymus nuclei were incubated with S-adenosyl-L- $\text{[}^{14}\text{C-methyl]}$ -methionine, ^{14}C was distributed in all the histone fractions, with lysine-rich histones incorporating radioactivity most actively, forming ϵ -N-methyllysine (18). ϵ -N-trimethyllysine was first detected in chicken erythrocyte histones (19). Avian erythrocyte histone F_3 contained all three forms (ϵ -N-monomethyllysine, ϵ -N-dimethyllysine, ϵ -N-trimethyllysine) of ϵ -N-methylated lysine (19). Delange *et al* (20) also demonstrated that all three forms of the ϵ -N-methyllysine were present in calf thymus F_3 histone.

Byvoet reported the presence of ϵ -N-methyllysine, 3-methylhistidine and ω -N-methylarginine from histones (combined arginine and slightly lysine-rich) prepared from the liver, thymus and subcutaneously transplanted Novikoff hepatoma of rat (21). Paik and Kim also reported the presence of ϵ -N-methylarginine in rat brain histone (22). In studying the histone methylation during the HeLa S-3 cell cycle, Paik *et al* (18) reported that the arginine-rich histones (F_{2a1} and F_3) were methylated at the highest rates, the slightly lysine-rich histones (F_{2b} and F_{2a2}) were methylated at lower rates and the lysine-rich histones (F_1) were not methylated at all. The F_3 polypeptides contained significant amounts of methylated arginine and histidine. Shepard, Hardin and Noland in studying the methylation of lysyl residues of histone in synchronized mammalian cells, reported that histone fraction F_1 was not methylated (23).

Duerre and Lee also reported F_1 histone isolated from an in vivo system did not contain any methylated basic amino acids (10). By incubating freshly prepared rat brain and liver nuclei with S-adenosyl-L- $[^3\text{H-methyl}]$ -methionine, Duerre and Lee (11) showed that the F_{2a1} and F_3 histones were the only components found to be highly methylated. After hydrolysis of brain and liver histones, the products of methylation in the F_{2a1} and F_3 histones were chiefly ϵ -N-monomethyllysine and ϵ -N-dimethyllysine. No methylated arginine or histidine was found in any of the histones analyzed.

Methylation is a very specific phenomenon. Delange, Smith, Fambrough and Bonner demonstrated that only 1 lysyl residue out of 11 in calf thymus F_{2a1} histone was methylated (4). Delange et al (20) also showed that both lysine residues 9 and 27 of calf thymus F_3 were partially ϵ -N-methylated. All three ϵ -N-methyl derivatives, ϵ -N-monomethyllysine, ϵ -N-dimethyllysine, and ϵ -N-trimethyllysine were present in the F_3 histone, while only ϵ -N-monomethyllysine and ϵ -N-dimethyllysine were present in F_{2a1} . From studies on the methylation of histones during the life cycle of the Chinese hamster ovary cells, Shepherd and coworkers (23) found that the ratio of ϵ -N-monomethyllysine to ϵ -N-dimethyllysine in the F_{2a1} , F_{2b} and F_3 histones varied from one phase to another. Borun and coworkers (24) have reported that 80-100% of all F_{2a1} , F_{2b} and F_3 polypeptides from Hela cells were methylated. The ratio of ϵ -N-dimethyllysine to ϵ -N-monomethyllysine in the heteroploid malignant Hela S-3 cell were about half the ratios of these amino acids found in the corresponding histones of normal cells. Desai

and Foley had compared the composition and structure of histone F_{2a1} from normal and neoplastic cells (25). They found that F_{2a1} from both sources was similar in overall structure, but the ratio of ϵ -N-monomethyllysine to ϵ -N-dimethyllysine was different.

Methylation of histones also appears to be dependent on age. In vitro experiments designed to measure the extent of methylation of various histone components of brain and liver during the aging process were carried out in this laboratory by Duerre and Lee (11). There was an increase in methylation of the histones in brain nuclei from birth until 11 days, then activities decreased progressively throughout the life span of the animal. In rat liver, methylase activity remained relatively constant up to 21 days of age, then gradually decreased. It was found that the extent of histone methylation was 3-4 times greater in brain than in liver during the developmental stages, while the extent of methylation was about the same in brain and liver nuclei from animals that were $2\frac{1}{2}$ years old. The F_3 and F_{2a1} histones were the only components found to be highly methylated and the degree of F_3 methylation was always higher than that of F_{2a1} . The products of methylation in the F_{2a1} and F_3 histones were mainly monomethyllysine and dimethyllysine. No methylated arginine or histidine was found. In all the histones the level of monomethyllysine was higher than that of dimethyllysine. Duerre and Lee (11) also found that the histone methylase activity was considerably higher in extracts prepared from brain nuclei of young animals as compared to old animals. Histones prepared from the

brains of old animals were poorer acceptors of methyl groups than histones prepared from brain of young animals. Histone methylase activity was also higher in extracts prepared from liver nuclei of young animals than old animals. However, there was no significant difference in the ability of histones prepared from liver nuclei of young and old animals to accept methyl groups.

MATERIALS AND METHODS

Forty-six newborn Sprague-Dawley albino rats were given 5 μ curies of L- ^3H -lysine and $2\frac{1}{2}$ μ curies of L- ^{14}C -methyl- ^3H -methionine per gram of body weight by intraperitoneal injection. This procedure was repeated after 24 hours. The animals were killed by decapitation 14 days after the last injection. The cerebellum, cerebrum, kidney, liver, and thymus were dissected out and placed separately in 0.32 M sucrose containing 1.0 mM potassium phosphate buffer, pH 7.4 and 1.0 mM MgCl_2 . All operations were carried out at 4°C unless otherwise specified.

Preparation of Labelled Nuclei

The organs were minced, suspended in 10 to 15 volumes of 0.32 M buffered sucrose and homogenized with a Teflon type homogenizer with a 0.01 inch clearance. About 10 strokes were used. The crude homogenate was then filtered through 4 layers of cheese cloth and the nuclei sedimented by centrifugation at $800 \times g$ for 10 minutes in a horizontal centrifuge head. The crude nuclei were suspended in 0.32 M buffered sucrose and washed 3 times by repeated centrifugation at $800 \times g$.

Preparation of Chromatin

The nuclei from the various organs were lysed in 0.14 M NaCl

plus 0.01 M sodium citrate. The chromatin was sedimented by centrifugation at 10,000 x g for 10 minutes. The chromatin was suspended in the same salt solution and washed twice.

Fractionation of Histones

The five major histone components from the various chromatins were isolated by a modification of the procedure of Johns (26).

Extraction of F_1 Histones. -- The chromatin preparations from the different tissues were homogenized in 5% perchloric acid (4 ml of acid per gram of original tissue) for 2 minutes in a Waring blender operated at maximum speed. The homogenate was centrifuged for 10 minutes at 10,000 x g and the sediment was extracted twice more in the same way. The supernatant fluid from the 3 extractions were combined and filtered through a grade 4 sintered glass funnel. The F_1 histones were precipitated by the addition of 3.3 M trichloroacetic acid to a final concentration of 1.1 M. The F_1 histones were recovered by centrifugation and washed once with acidified acetone (1,000 ml acetone + 0.5 ml HCl), followed by two washings in acetone. The F_1 histones were air dried and dissolved in 0.01 N HCl.

Extraction of F_3 and F_{2a} Histones. -- The sediment remaining after the extraction of F_1 histones was suspended in ethanol (2 ml/gm original tissue). The suspension was stirred extensively with a glass rod to break up all lumps. The suspension was let stand for 18 hours at 4°C and the sediment was removed by

centrifugation for 10 minutes at 10,000 x g. The sediment was extracted twice more with the same amount of 80% ethanol. The supernatant fluids containing the F_{2a} and F_3 histones were combined and the histones precipitated by the addition of 5 volumes of acetone followed by 0.5 ml of concentrated HCl per 100 ml of supernatant fluid. The histones were collected by centrifugation at 10,000 x g for 10 minutes, washed once in acetone and dissolved in water (about 20 mg/ml). Ethanol and 5 N HCl were added to make the solution 80% with respect to ethanol and 0.25 N with respect to HCl. The solution was dialyzed for 18 hours against 2 volumes of ethanol, followed by a second dialysis for 4 hours against 2 volumes of ethanol. The precipitated F_3 histone was collected by centrifugation, washed once with ethanol and three times with acetone, air dried and dissolved in 0.01 N HCl.

The F_{2a} histone remaining in the supernatant fluid was precipitated by the addition of 3 volumes of acetone. The precipitate was washed twice with acetone, air dried and dissolved in 0.01 N HCl.

Extraction of F_{2b} Histones. -- The F_{2b} histones remaining in the sediment, after the extraction of F_3 and F_{2a} histones with ethanol, were solubilized by homogenization in a Waring blender with 0.25 N HCl (2 ml/gm of tissue) at half speed. After centrifugation at 10,000 x g for 10 minutes the sediment was extracted twice more in the same way. The supernatant fluid from these extractions was combined and the F_{2b} histone precipitated with 5 volumes of acetone. The F_{2b} histone was washed twice with acetone, air dried and dissolved in 0.01 N HCl.

Purification of Histones by Gel Filtration

All the histones so prepared were further purified by gel filtration. A column of Bio-Gel P-10 (1.6 cm x 3.5 m) was sufficient for the purification of about 10 mg of histone. The F_3 histones were first reduced with dithiothreitol (DTT) before applying to the column. DTT (0.1 M) was added to the F_3 histones to a final concentration of 0.02 M. The histones were made 6.0 M with respect to urea, placed on the column and eluted with 0.01 N HCl. Elution was followed at 230 nm with a Beckman DB ratio recording spectrophotometer.

DEAE - Cellulose Chromatography

The lysine-rich histones (F_1) were further purified by DEAE-cellulose chromatography according to the procedure of Duerre and Gaitonde (27). DEAE-cellulose (Cellex-D) was washed twice with 0.5 N NaOH followed by several water washes. The DEAE-cellulose was placed on 1 x 20 cm columns and equilibrated with 50 ml of 0.01 M glycine-NaOH buffer, pH 9.7, followed by 25 ml of the same buffer containing 8 M urea. The histones (2.5 mg) were dissolved in the 0.01 M glycine-NaOH buffer containing 8 M urea and applied to the column. After passage of 50 ml of buffered urea solution through the column the effluent was free from protein. The effluent was pooled and dialyzed for 10 hours against 20 volumes of 0.2 N HCl, followed by dialysis against 20 volumes of 1.0 mM HCl for 24 hours with five changes. The protein was recovered by freeze-drying and dissolved in 0.01 N HCl.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis, based on the method of Panyim and Chalkley (28), was used to check the purity of the histone components.

Acrylamide, N,N'-bisacrylamide and N,N,N',N',-tetramethylethylenediamine (TEMED) were purchased from Eastman Chemicals. Acrylamide gel formation requires the mixing of acrylamide with polymerization accelerator and catalyst, all of which were stored in separate containers. The following solutions were mixed to give a 15% polyacrylamide gel in 6.25 M urea at a final pH of 3.2. Two parts of Solution A (Appendix) were mixed with 1 part of Solution B (Appendix) and 5 parts of Solution C (Appendix).

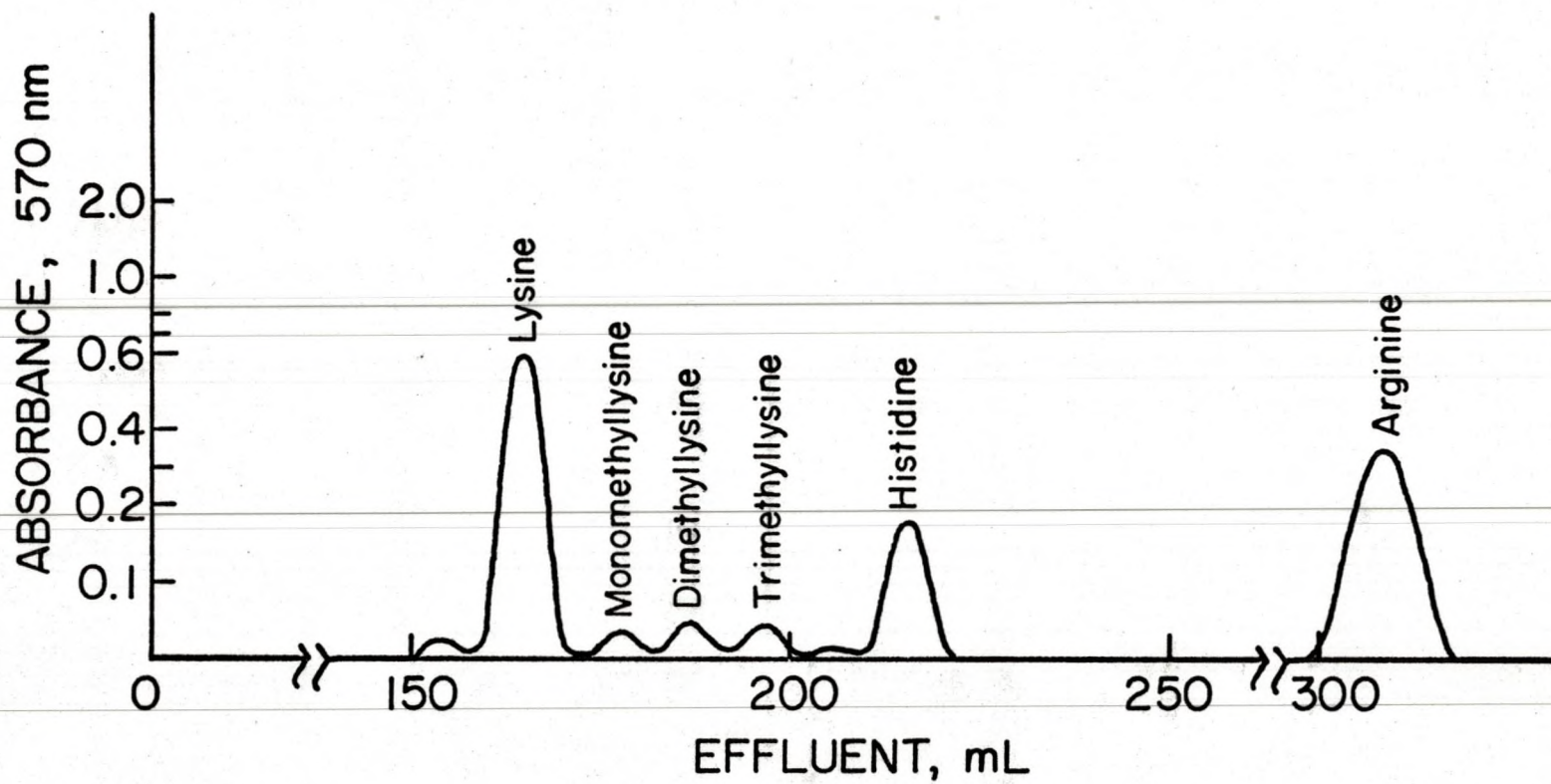
After polymerization the gels were pre-electrophoresed to remove all charged species (other than proton and acetate ions) for 4 hours before the histone samples were applied to the gels. Electrophoresis was performed at room temperature in a standard vertical gel system, using tubes of 0.8 cm in diameter and 10 cm in length for 4½ hours. The tray buffer was 0.9 N acetic acid. About 10 µg of histone was mixed with a drop of dye plus sugar (Appendix) and applied to the gel. A Beckman Duostat regulated D.C power supply was used to provide a constant current. The current was maintained at 2 mA per tube of gel. The gels were removed from their glass tubes by rimming the tubes with a fine jet of water from a hypodermic needle, as described by Reisfeld et al (29), and stained overnight in the staining solution (Appendix). Destaining was accomplished by washing the gels in 7% acetic acid.

Basic Amino Acid Analysis

Quantitative analysis of the basic amino acids of the histone hydrolysates was performed with the aid of a Technicon automatic amino acid analyzer. Each of the histone components (2-3 mg) was hydrolyzed in 6 N HCl for 24 hours at 110°C in vacuo. The histone sample was made 6 N with respect to HCl in a 10 ml round-bottom flask. The sample was frozen by rotating the flask in an alcohol dry-ice bath and the flask was hooked onto a vacuum pump. When the flask was under vacuum, nitrogen was flushed into the flask. The sample was thawed and the process repeated three times. The flask was then sealed under vacuum and put into an oil bath at 110°C for 24 hours.

The hydrolysates were evaporated to dryness with a Rotovac flash evaporator and the process repeated three times by the addition and removal of water. This process removed all the HCl that was present in the histone hydrolysate. The hydrolysate was dissolved in 0.3 ml of 0.2 M sodium citrate buffer, pH 2.2, and applied to a column (0.9 x 40 cm) of Beckman PA-35 resin. The column was eluted with 0.35 M citrate buffer, pH 5.82, at 26°C under 350 psi. The stream from the column was split with a divider with half of the effluent passing through the analyzer while the other half was collected with the aid of a fraction collector over 10 minute intervals under peaks of lysine, ϵ -mono, di and trimethyllysine. At all other time fractions of 30 minute intervals were collected. Figure 1 shows the elution profile of a standard amino acid sample containing 1 μ mole each of lysine, histidine, arginine and 0.3 μ mole each of monomethyl-

Fig. 1.--Elution profile of a standard amino acid sample containing 1 μ mole each of lysine, histidine, arginine and 0.3 μ mole each of monomethyllysine, dimethyllysine and trimethyllysine on Beckman PA-35 resin. The column (0.9 x 40 cm) was eluted with 0.35 M citrate buffer, pH 5.82 at 26°C. The flow rate was 27 ml/hour. The stream from the column was divided and one half of the effluent passed through a Technicon automatic amino acid analyzer, the remaining effluent was collected in 10 minute fractions.



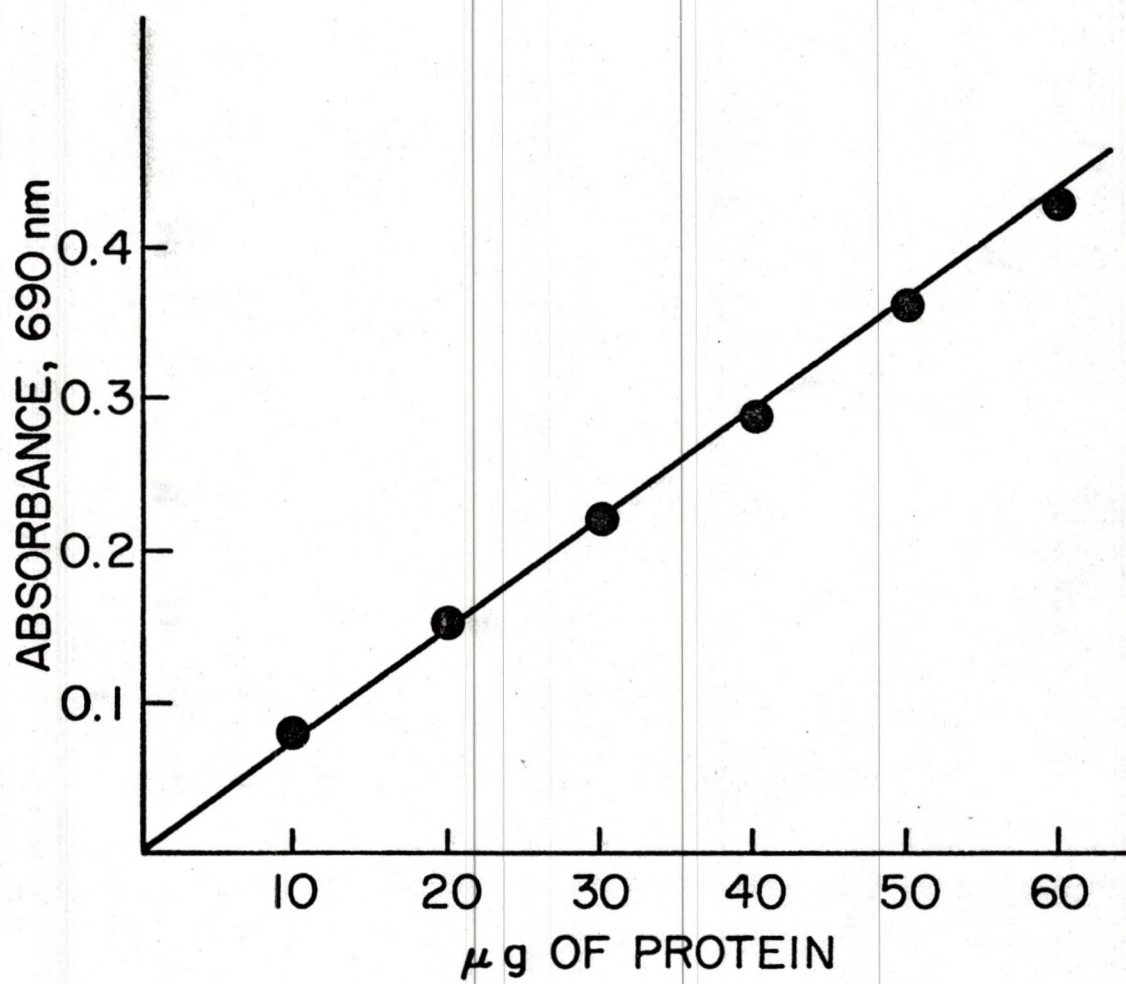
lysine, dimethyllysine and trimethyllysine.

Radioactivities were used to determine the distribution of the ^{14}C -methyl groups in the basic amino acids residing in the polypeptide chain. This was accomplished by counting 1 ml aliquots of the fractions collected in Bray's counting fluid (Appendix) in a Packard Tri-carb scintillation spectrometer. Quenching due to salt and water was corrected for by channels ratio technique using both internal and external standards. Radioactivity was expressed as counts per minute per μmole .

Determination of Protein

The method of Lowry, Rosebrough, Farr and Randall (30) was used to determine the protein concentration of all histone fractions. Sample of the histone fractions containing between 10 and 60 μg protein were diluted to a volume of 0.6 ml with water. Three ml of Reagent C (Appendix) were added. The solution was mixed and allowed to stand at room temperature for 10 minutes. For color development 0.3 ml of Folin-Ciocalteu Phenol reagent (1.0 N with respect to H_2SO_4) was added with immediate and rapid mixing. After standing for 30 minutes at room temperature the samples were read in a Coleman Jr. spectrophotometer at 690 nm against a reagent blank. A standard curve using Bovine serum albumin was found to be linear with protein concentration between 10 and 60 μg per ml (Figure 2).

Fig. 2.--Reference curve for the determination of protein by the method of Lowry et al (30). Bovine serum albumin was used as the standard solution.



Determination of the Relative Distribution of Histone Components

Crude histones from the cerebellum, cerebrum, liver, thymus and kidney were extracted according to the procedure of Duerre and Gaitonde (27) from thirty 12 day old rats. The chromatins were prepared as described previously. The chromatins were extracted twice with methanol-chloroform (1:2) to remove lipids, particularly myelin from the cerebellum and cerebrum. This procedure also denatures proteolytic enzymes that might be present. The chromatins were then washed 2 times with acetone to remove any residual chloroform; followed by 2 washes with acetic acid pH 3. The histones were extracted from the residue with 5 volumes of 0.4 N HCl. After centrifugation at 30,000 x g for 10 minutes, the supernatant fluid was collected and the crude histones precipitated by the addition of 5 volumes of acetone. The histones were recovered by centrifugation, air dried and dissolved in 0.01 N HCl.

Twenty-five μ g of total crude histone from the various organs were applied to polyacrylamide gels and electrophoresis was carried out as described previously. The relative per cent of the various bands on the gels were determined by scanning the gels through a Helena Quick Scan densitometer.

RESULTS

Polyacrylamide gel electrophoresis was carried out on the total histone extracted from the rat cerebellum, cerebrum, liver, thymus and kidney according to the procedure of Duerre and Gaitonde (27). The F_{2a2} and F_{2b} histones moved close together under the conditions employed (Figure 3). The F_3 histones were separated into 2 bands, the oxidized form (S-S) migrated much slower than the reduced form (S-H). It was found that 25 μ g of histones applied to the gels gave the best resolution. Figure 3 shows that the histones extracted were contaminated slightly with non-histone proteins. However, these proteins did not interfere with the quantitation of the histone components. These proteins could be removed by passing the histones through DEAE cellulose as described under Materials and Methods. However, poorer resolution was obtained on the gels. The various histone bands became more diffused into the gels. Table 1 summarizes the relative distribution of histone components from the various organs. There was no significant difference in the distribution of the various histones from organ to organ.

Before measuring the distribution of methylated amino acid residues in various histone components, the histones were purified on Bio-Gel P-10. Figures 4-8 show the elution profile

Fig. 3.--Polyacrylamide gel electrophoresis of the total histones extracted from rat cerebellum, cerebrum, liver, thymus and kidney according to the procedure of Duerre and Gaitonde (27). 25 μ g of each of the total histone was applied to the gels and electrophoresis was carried out as described under Materials and Methods.

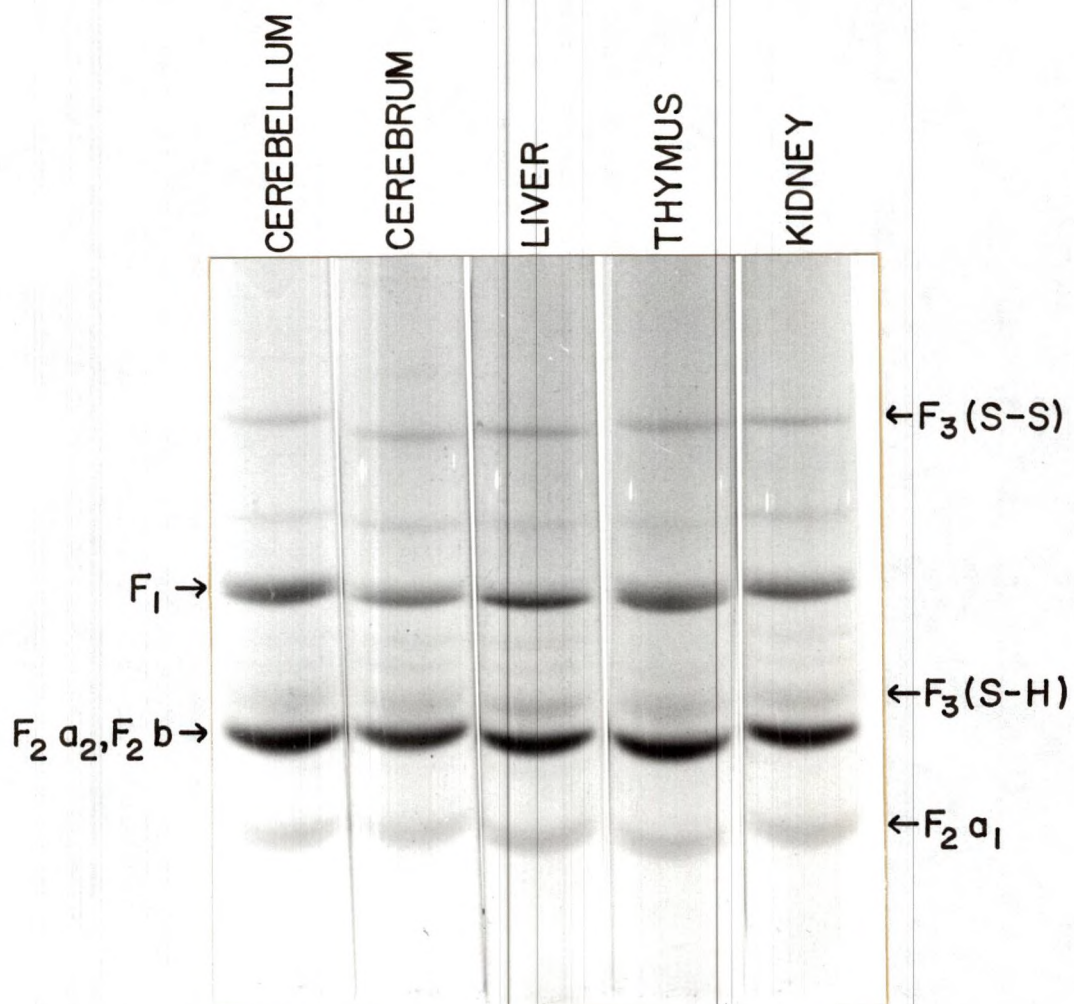


TABLE 1
THE DISTRIBUTION OF HISTONE FRACTIONS
FROM THE VARIOUS ORGANS OF THE RAT

ORGAN	RELATIVE PER CENT			
	F ₁	F ₃	F _{2a2} + F _{2b}	F _{2a1}
CEREBELLUM	24.9	14.5	46.1	14.5
CEREBUM	21.7	22.3	42.7	13.3
LIVER	20.0	16.3	46.1	17.6
THYMUS	21.8	15.8	45.9	16.5
KIDNEY	18.7	14.9	47.1	19.3

Chromatins from the cerebellum, cerebrum, liver, thymus and kidney were prepared from thirty 12 day old rats as described under Material and Methods. The crude total histones were extracted according to the procedure of Duerre and Gaitonde (27). Twenty-five μ g of total histone from each organ was applied to polyacrylamide gel and electrophoresis was carried out as described under Material and Methods. The relative per cent of the various bands on the gels were determined by scanning the gels through a Helena Quick Scan densitometer.

Fig. 4.--Elution profile of rat cerebellum histones on Bio-Gel P-10. The histone fractions extracted by the procedure of Johns (26) were made 6 M with respect to urea, placed on the column and eluted with 0.01 N HCl. Elution was followed at 230 nm with a Beckman DB ratio recording spectrophotometer. The F_{2a} histone was further fractionated into F_{2a2} and F_{2a1} . The proteins under the various peaks were concentrated by lyophilization for further analysis.

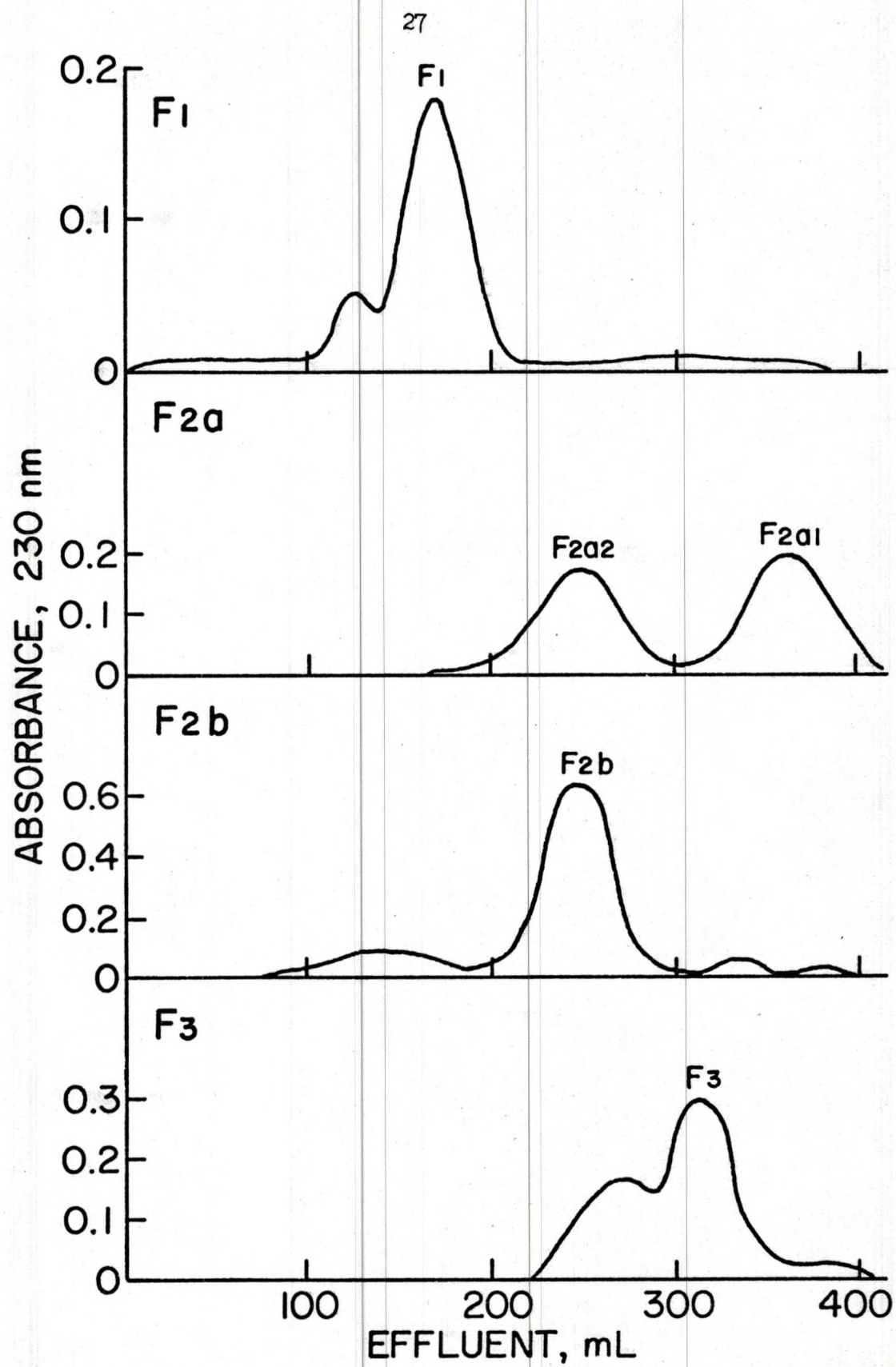


Fig. 5.--Elution profile of rat cerebrum histones on Bio-Gel P-10. The histone fractions extracted by the procedure of Johns (26) were made 6 M with respect to urea, placed on the column and eluted with 0.01 N HCl. Elution was followed at 230 nm with a Beckman DB ratio recording spectrophotometer. The F_{2a} histone was further fractionated into F_{2a2} and F_{2a1} . The proteins under the various peaks were concentrated by lyophilization for further analysis.

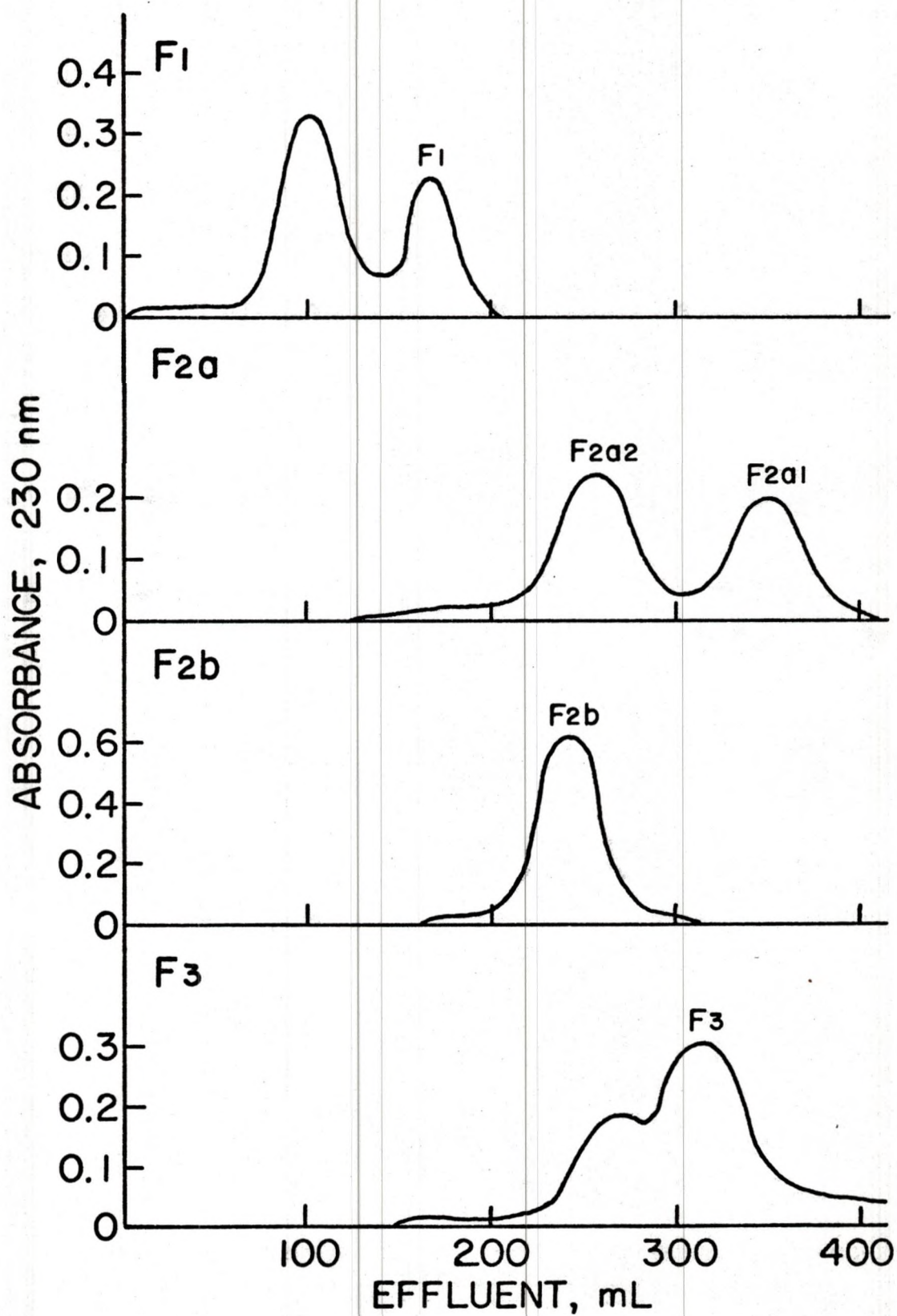


Fig. 6.--Elution profile of rat liver histones on Bio-Gel P-10. The histone fractions extracted by the procedure of Johns (26) were made 6 M with respect to urea, placed on the column and eluted with 0.01 N HCl. Elution was followed at 230 nm with a Beckman DB ratio recording spectrophotometer. The F_{2a} histone was further fractionated into F_{2a2} and F_{2a1} . The proteins under the various peaks were concentrated by lyophilization for further analysis.

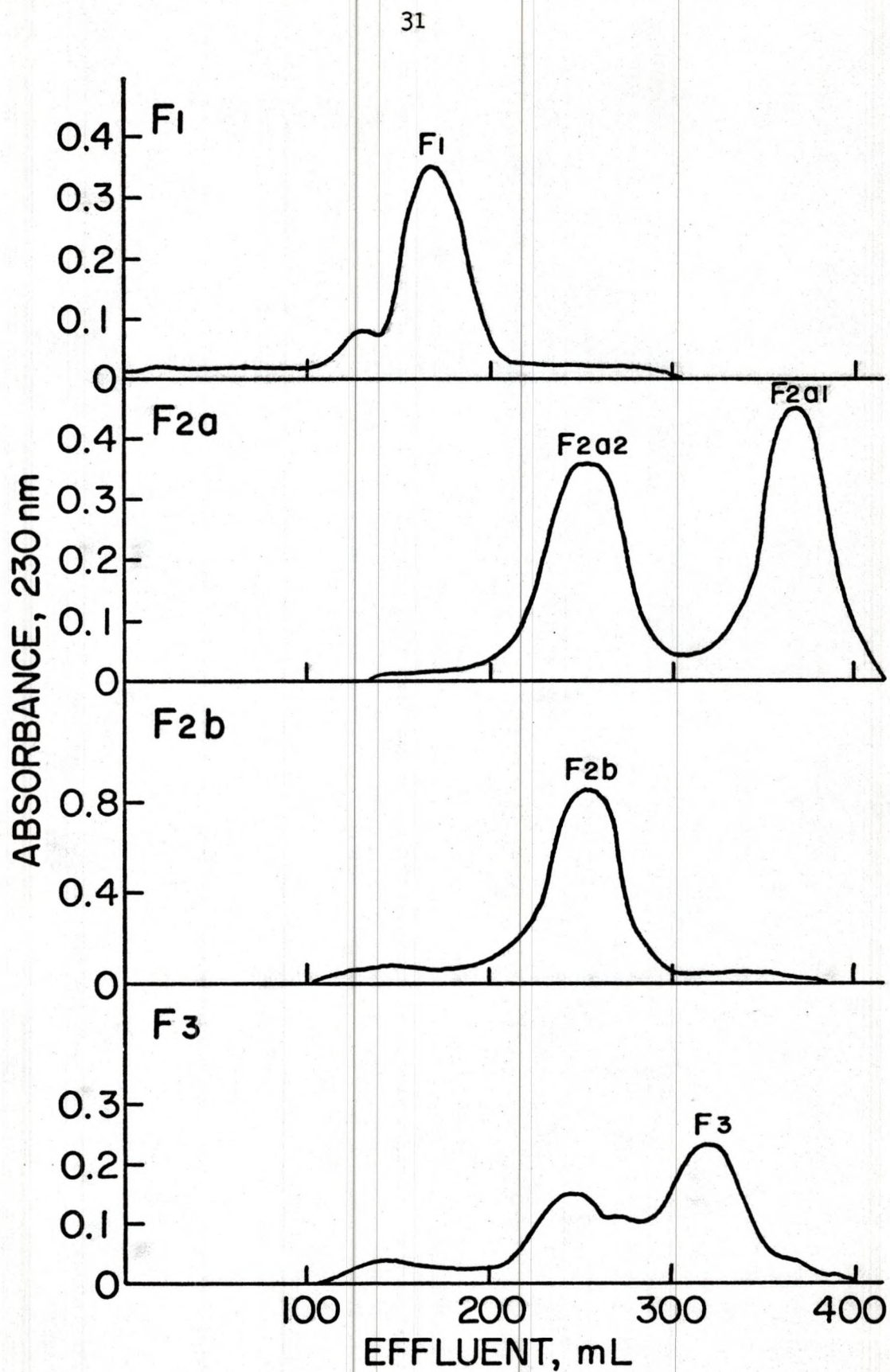


Fig. 7.--Elution profile of rat thymus histones on Bio-Gel P-10. The histone fractions extracted by the procedure of Johns (26) were made 6 M with respect to urea, placed on the column and eluted with 0.01 N HCl. Elution was followed at 230 nm with a Beckman DB ratio recording spectrophotometer. The F_{2a} histone was further fractionated into F_{2a2} and F_{2a1} . The proteins under the various peaks were concentrated by lyophilization for further analysis.

33

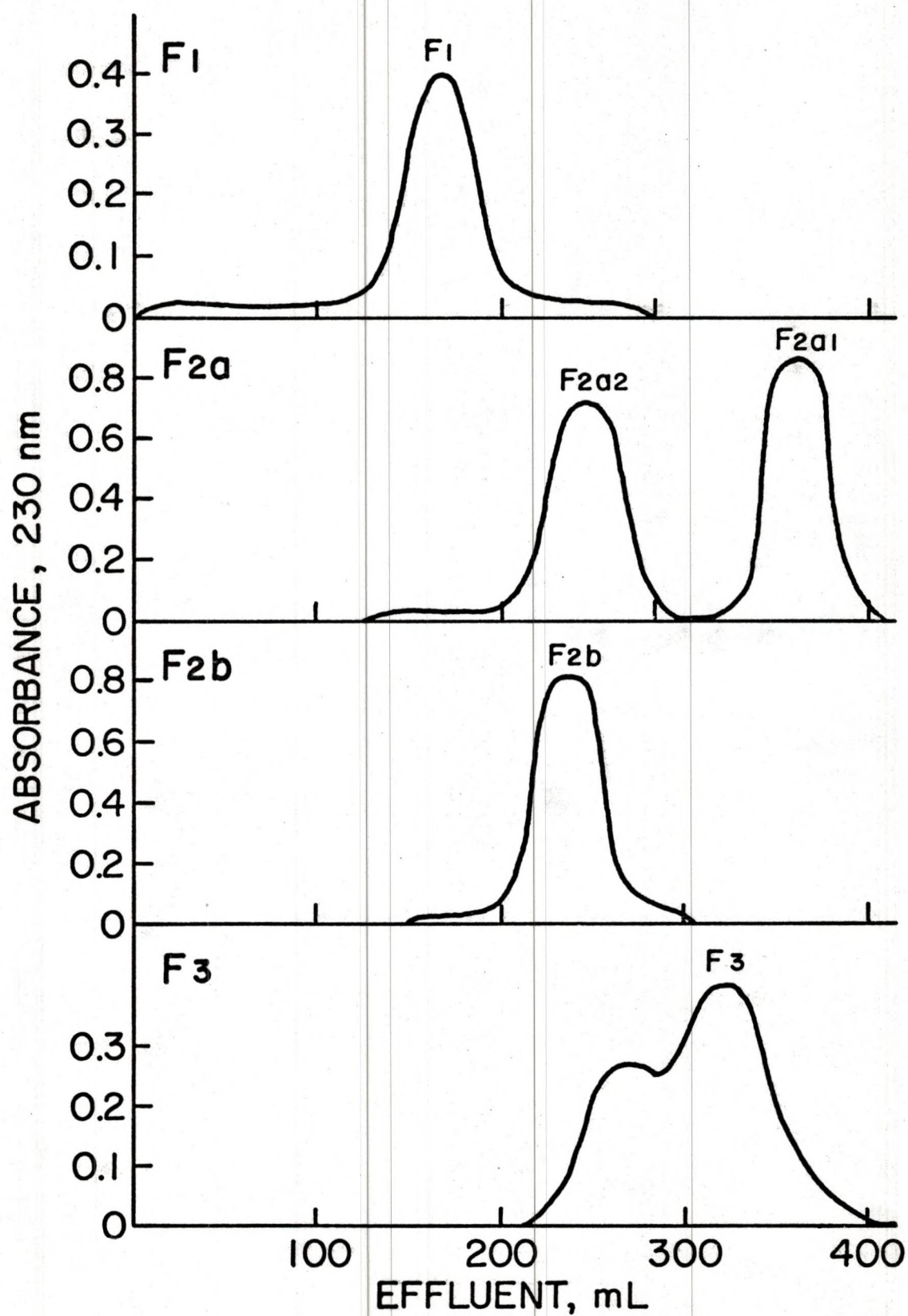
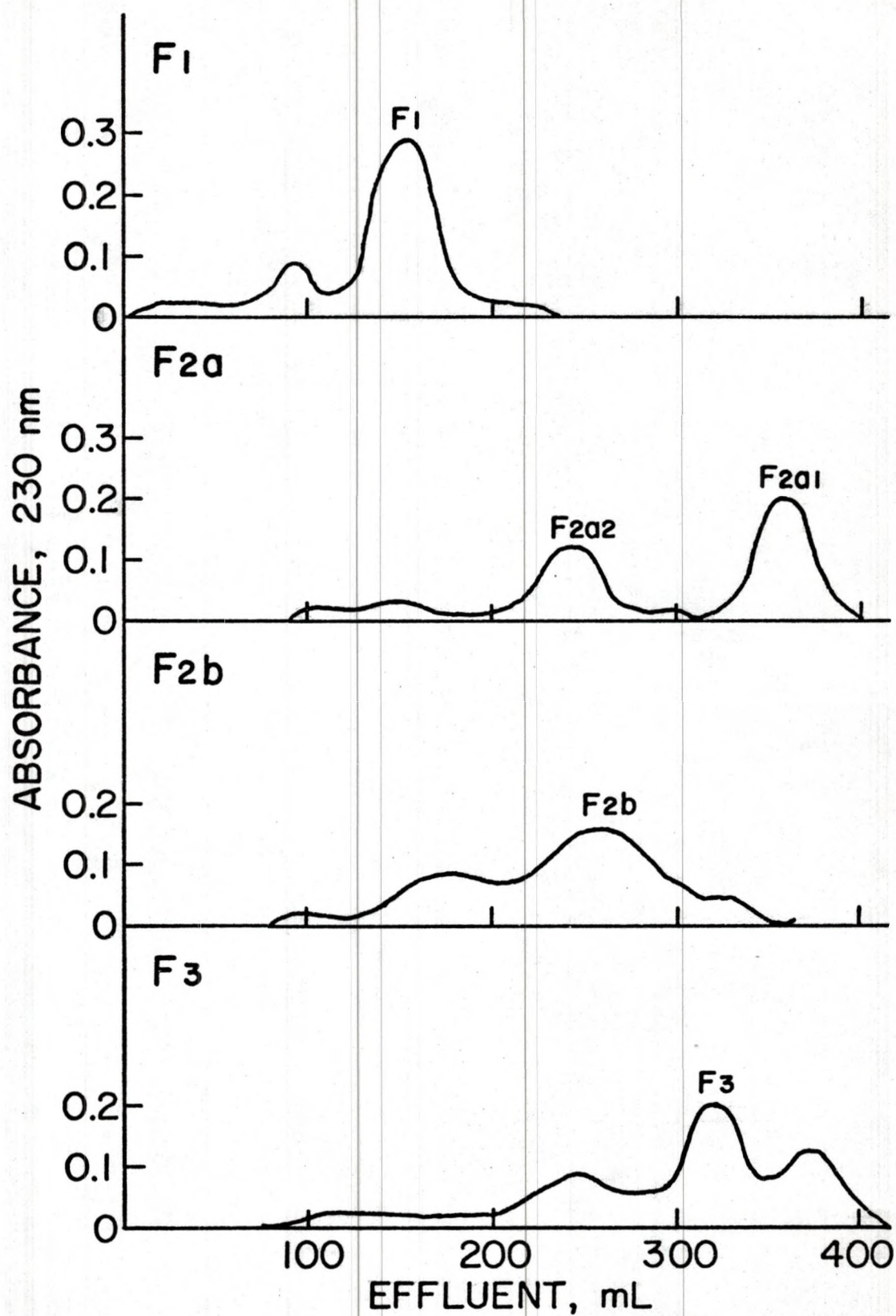


Fig. 8.--Elution profile of rat kidney histones on Bio-Gel P-10. The histone fractions extracted by the procedure of Johns (26) were made 6 M with respect to urea, placed on the column and eluted with 0.01 N, HCl. Elution was followed at 230 nm with a Beckman DB ratio recording spectrophotometer. The F_{2a} histone was further fractionated into F_{2a2} and F_{2a1} . The proteins under the various peaks were concentrated by lyophilization for further analysis.

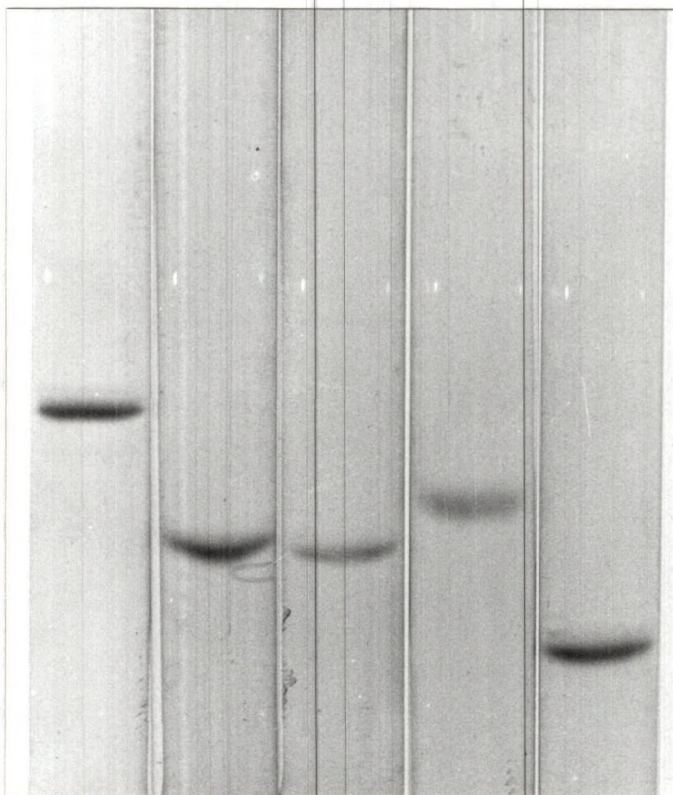


on Bio-Gel P-10 of the various isotopically labelled histones prepared from the cerebellum, cerebrum, liver, thymus and kidney of rats by the method of Johns (26). The F_{2a2} and F_{2b} histones eluted so close together from the Bio-Gel P-10 that they were inseparable on the basis of molecular weights. The F_{2a} histones were further fractionated into F_{2a2} and F_{2a1} . The proteins under the various peaks were concentrated by lyophilization and their purity checked by polyacrylamide gel electrophoresis (Figures 9-13). Most of the F_3 histones were found to be contaminated with F_{2a2} or F_{2b} after passing through Bio-Gel P-10. Purification was achieved by repeated gel filtration after which quantities available did not allow for a complete chemical analysis of the basic methylated amino acid residues. After passage through Bio-Gel the F_1 histones were further purified by DEAE cellulose. After passage through DEAE they were devoid of $[I^{14}C]$ -carbon. Since all the F_1 histones were found to be devoid of any methylated basic amino acid residues or methionine, the absence of $[I^{14}C]$ -carbon could also be used to assess purity. The F_{2a2} , F_{2b} and F_{2a1} histones were pure after passage through Bio-Gel.

The purified histones were hydrolyzed and the basic amino acids were fractionated on a column of Beckman PA-35 resin with half of the effluent passing through an automatic amino acid analyzer as described under Materials and Methods. Figures 14-18 show the elution profile of the basic amino acids from the 5 histone components (F_1 , F_{2a2} , F_{2b} , F_{2a1} and F_3) from rat thymus.

Fig. 9.--Polyacrylamide gel electrophoresis of the five histone components from rat cerebellum that were fractionated on Bio-Gel P-10 (Figure 4).

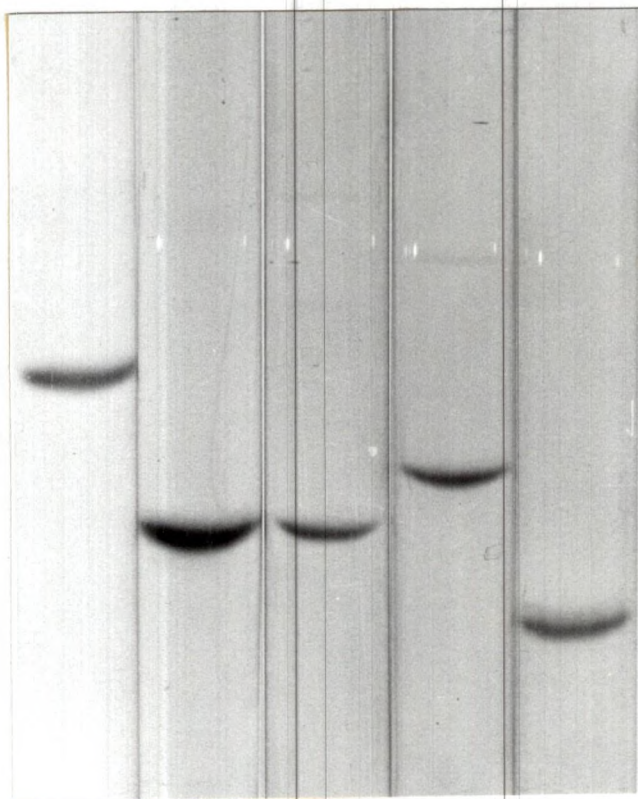
F₁ F_{2a2} F_{2b} F₃ F_{2a1}



CEREBELLUM

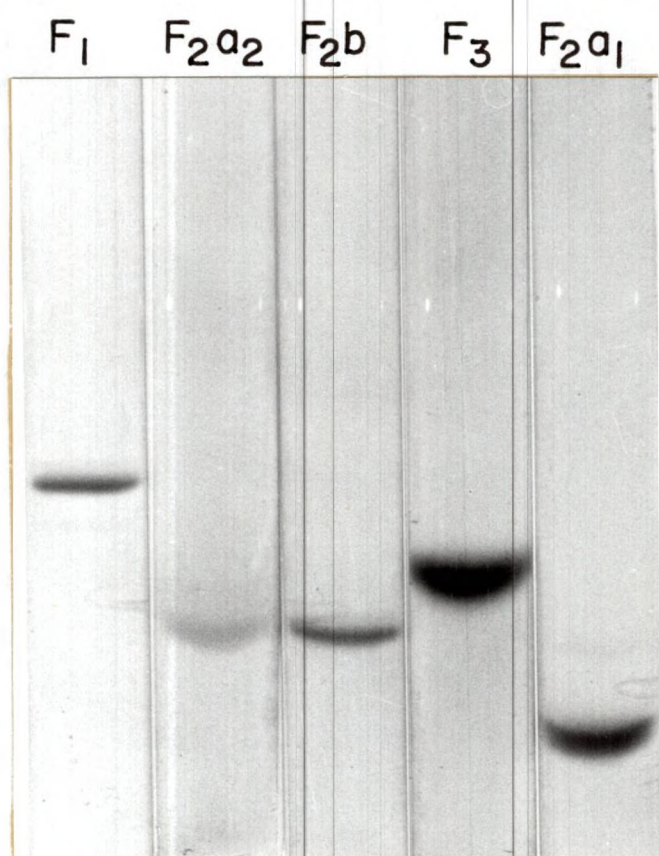
Fig. 10.--Polyacrylamide gel electrophoresis of the five histone components from rat cerebrum that were fractionated on Bio-Gel P-10 (Figure 5).

F₁ F₂a₂ F₂b F₃ F₂a₁



CEREBRUM

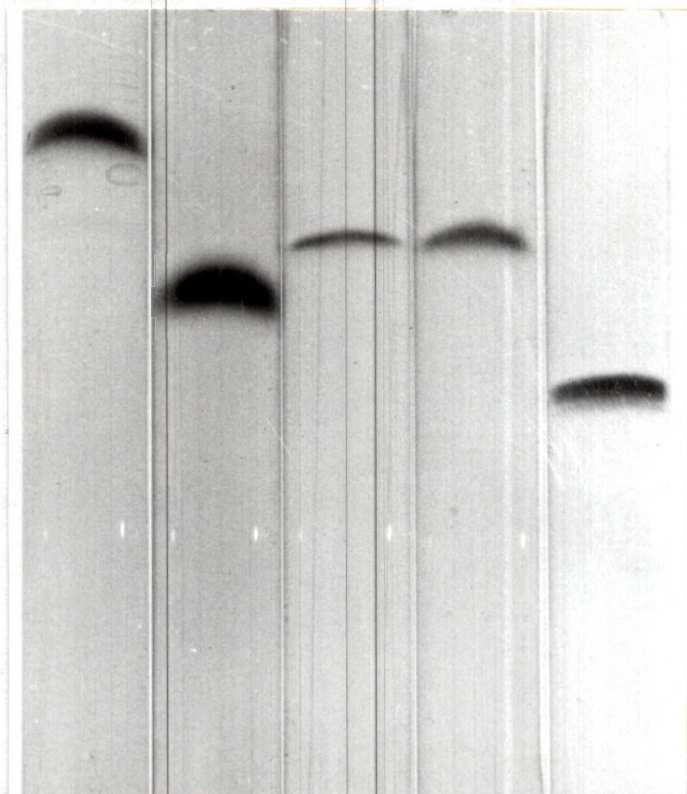
Fig. 11.--Polyacrylamide gel electrophoresis of the five histone components from rat liver that were fractionated on Bio-Gel P-10 (Figure 6).



LIVER

Fig. 12.--Polyacrylamide gel electrophoresis of the five histone components from rat thymus that were fractionated on Bio-Gel P-10 (Figure 7).

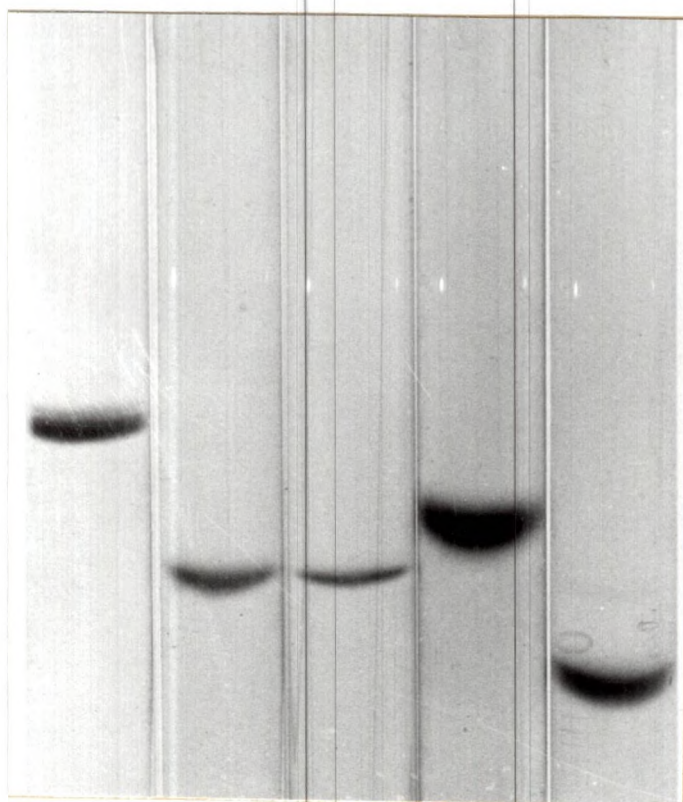
THYMUS



F1 F2a2 F2b F3 F2a1

Fig. 13.--Polyacryalmide gel electrophoresis of the five histone componenets from rat kidney that were fractionated on Bio-Gel P-10 (Figure 8).

F_1 F_2a_2 F_2b F_3 F_2a_1



KIDNEY

Fig. 14.--Fractionation of the basic amino acids from rat thymus F₁ histone on Beckman PA-35 resin. The column (0.9 x 40 cm) was eluted with 0.35 M citrate buffer, pH 5.82 at 26°C. The flow rate was 27 ml/hour. The stream from the column was divided and one-half of the effluent passed through a Technicon automatic amino acid analyzer, the remaining effluent was collected in 10 minute fractions. Radioactivity was determined on these fractions using a Packard tricarb scintillation counter.

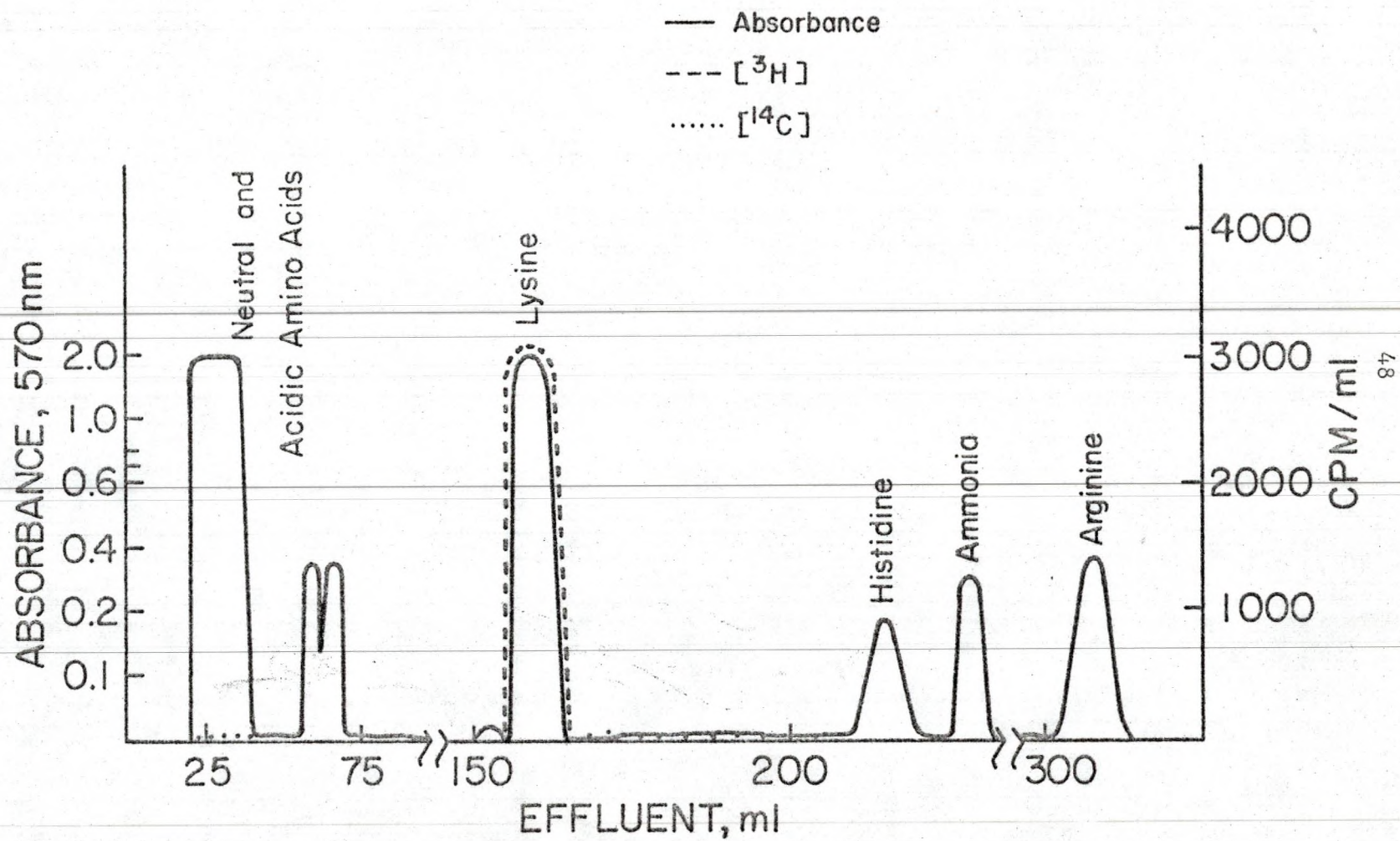


Fig. 15.--Fractionation of the basic amino acids from rat thymus F_{2a2} histone on Beckman PA-35 resin. The column (0.9 x 40 cm) was eluted with 0.35 M citrate buffer, pH 5.82 at 26°C. The flow rate was 27 ml/hour. The stream from the column was divided and one-half of the effluent passed through a Technicon automatic amino acid analyzer, the remaining effluent was collected in 10 minute fractions. Radioactivity was determined on these fractions using a Packard tricarb scintillation counter.

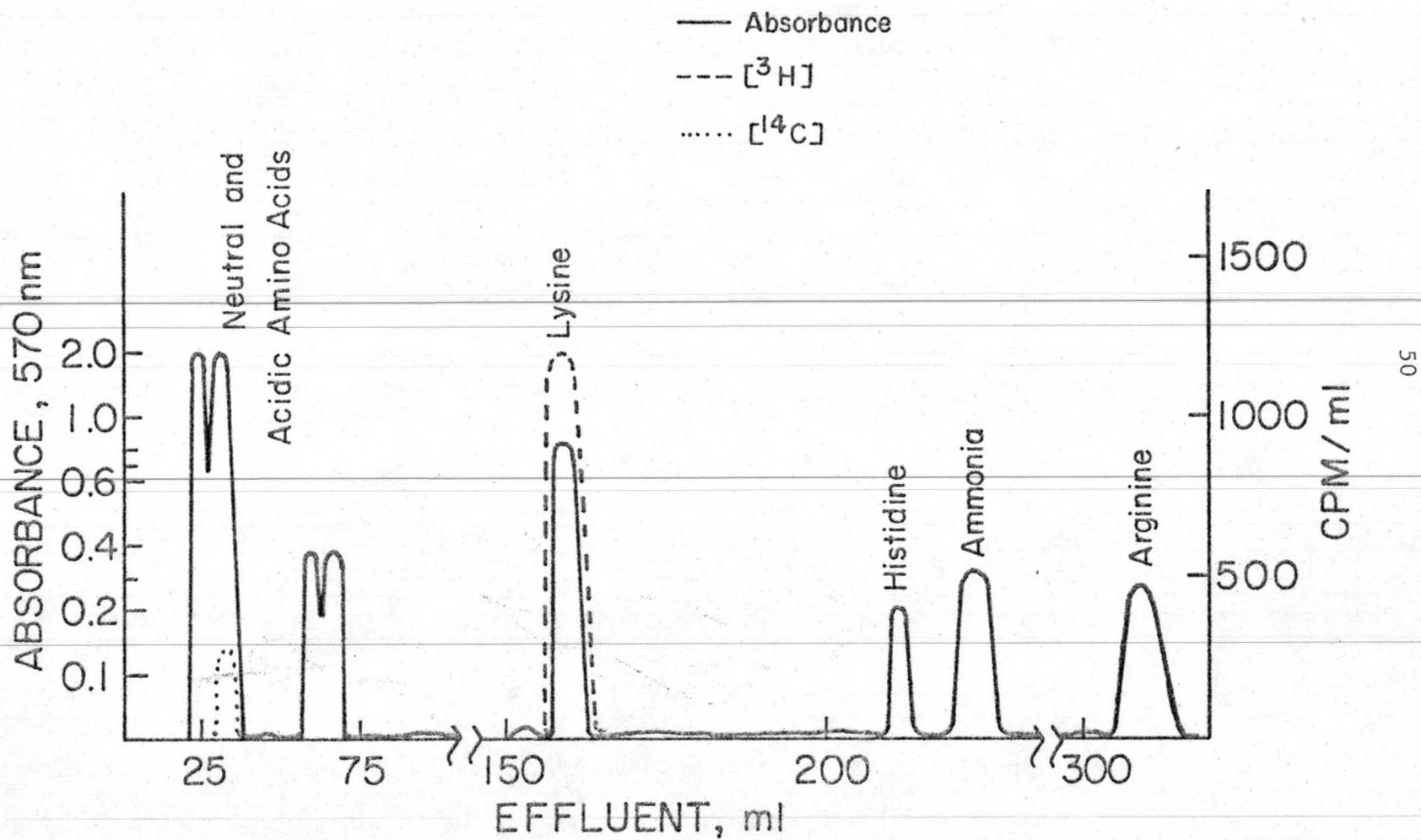


Fig. 16.--Fractionation of the basic amino acids from rat thymus F_{2b} histone on Beckman PA-35 resin. The column (0.9 x 40 cm) was eluted with 0.35 M citrate buffer, pH 5.82 at 26°C. The flow rate was 27 ml/hour. The stream from the column was divided and one-half of the effluent passed through a Technicon automatic amino acid analyzer, the remaining effluent was collected in 10 minute fractions. Radioactivity was determined on these fractions using a Packard tricarb scintillation counter.

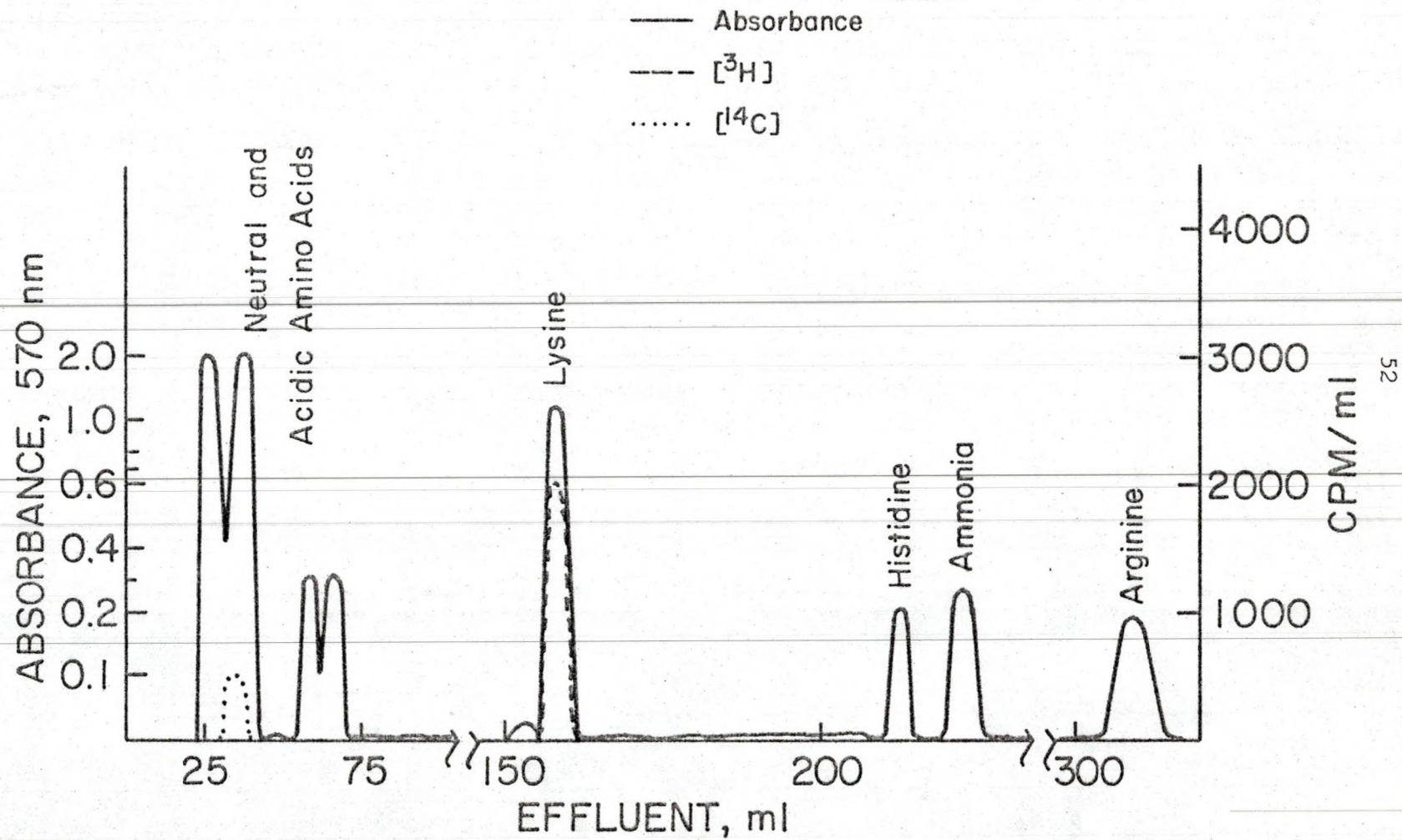


Fig. 17.--Fractionation of the basic amino acids from rat thymus F_{2a1} histone on Beckman PA-35 resin. The column (0.9 x 40 cm) was eluted with 0.35 M citrate buffer, pH 5.82 at 26°C. The flow rate was 27 ml/hour. The stream from the column was divided and one-half of the effluent passed through a Technicon automatic amino acid analyzer, the remaining effluent was collected in 10 minute fractions. Radioactivity was determined on these fractions using a Packard tricarb scintillation counter.

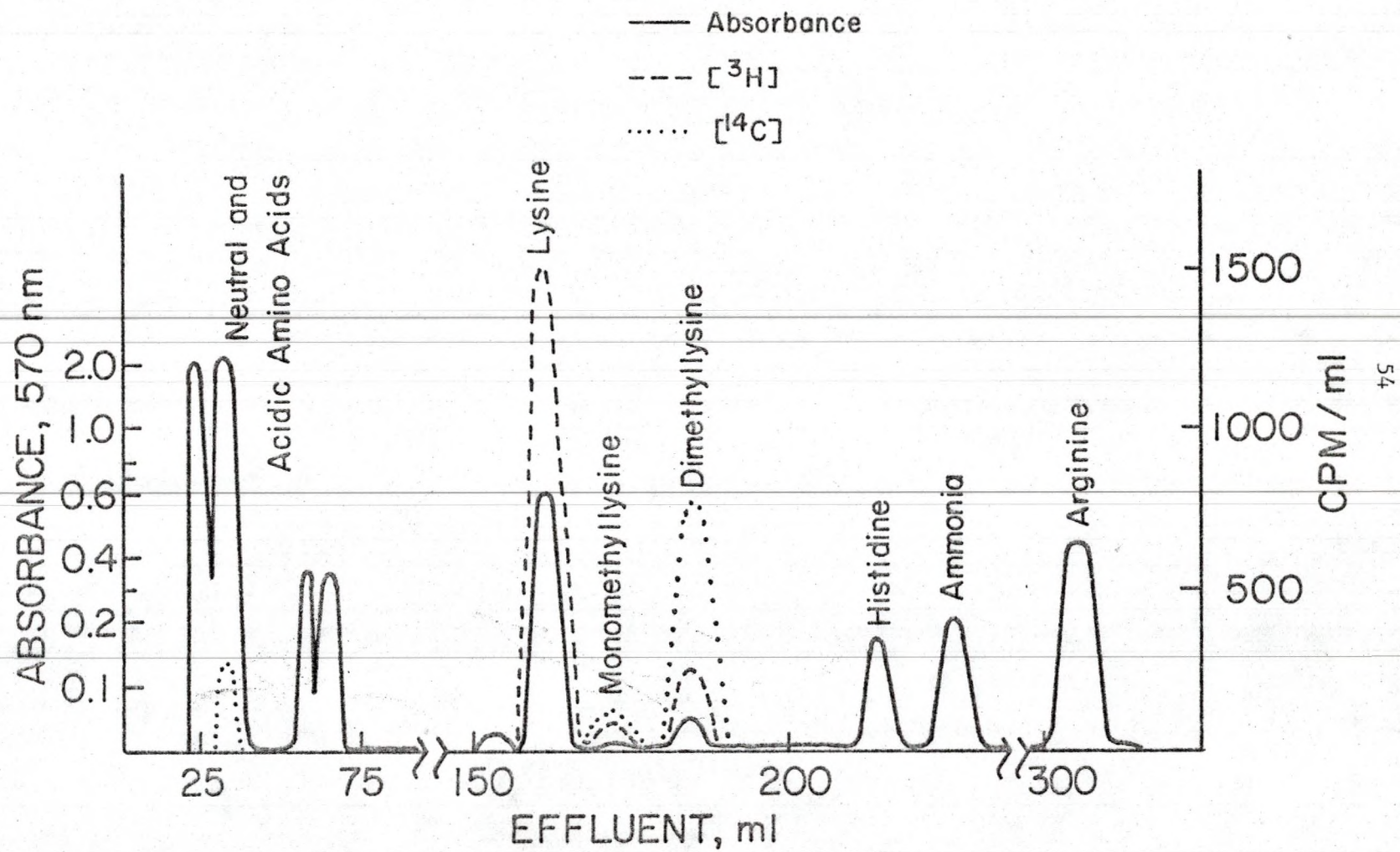
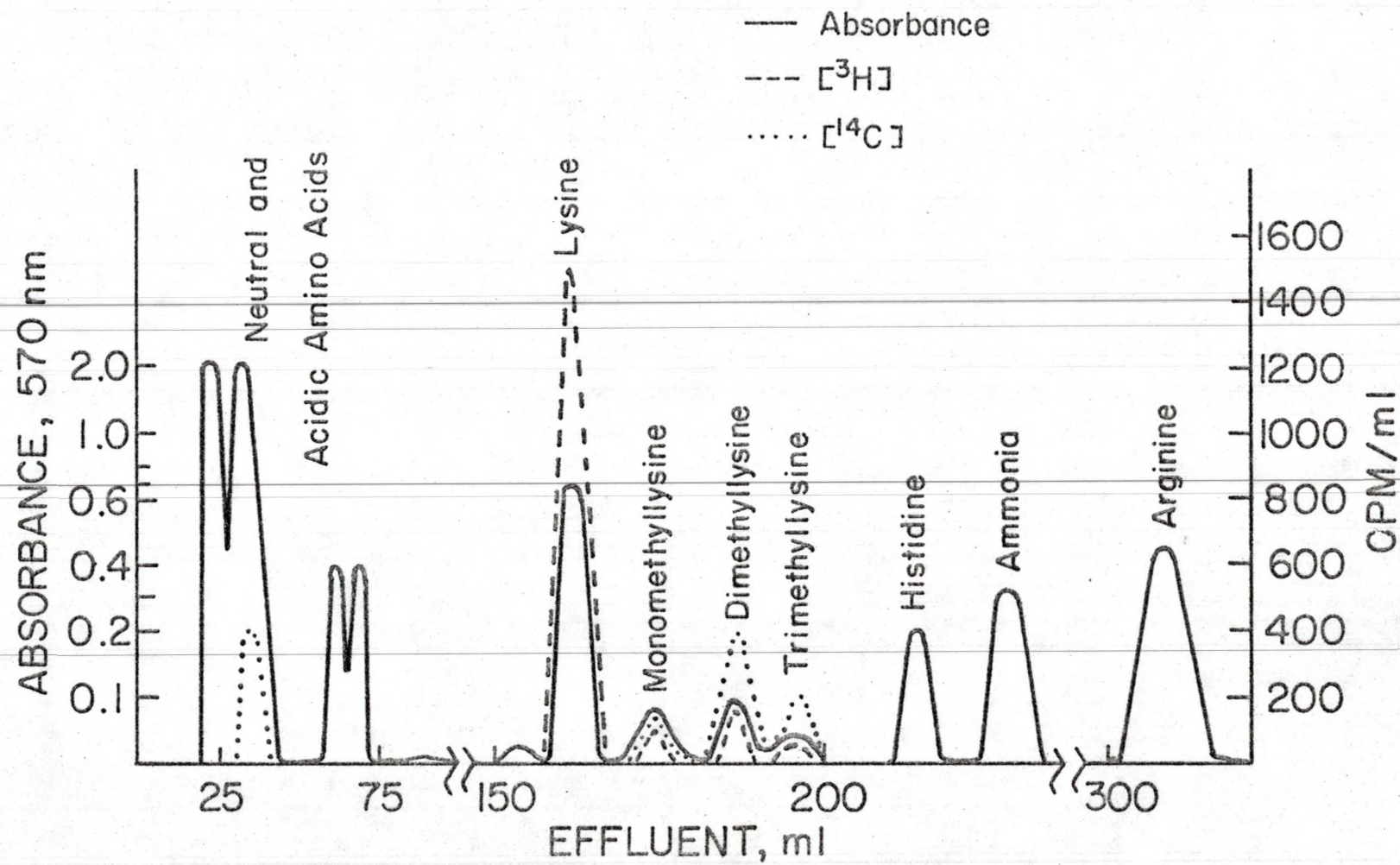


Fig. 18.--Fractionation of the basic amino acids from rat thymus F₃ histone on Beckman PA-35 resin. The column (0.9 x 40 cm) was eluted with 0.35 M citrate buffer, pH 5.82 at 26°C. The flow rate was 27 ml/hour. The stream from the column was divided and one-half of the effluent passed through a Technicon automatic amino acid analyzer, the remaining effluent was collected in 10 minute fractions. Radioactivity was determined on these fractions using a Packard tricarb scintillation counter.



The elution profile of the basic amino acids from the histones of the other organs were essentially the same. No methylated amino acids were found in any of the F_1 histones analyzed.

Tritium was detectable only in the lysine peak and no radioactivity, either ^3H or ^{14}C , was detected in the regions of the methylated amino acid residues (Figure 14).

The F_{2a2} histones from all of the organs studied were also devoid of any methylated amino acids. Tritium was detectable only in the lysine peak and ^{14}C was found under the first peak where methionine was eluted. No radioactivity, either ^3H or ^{14}C , was detected in the regions of methylated amino acid residues (Figure 15). The profiles of the F_{2b} histones were essentially the same as that of the F_{2a2} histones (Figure 16). However, traces of radioactivity both ^3H and ^{14}C , were detected in the regions of ϵ -N-mono and dimethyllysine. Neither of these methylated amino acids were in high enough concentration to be detectable by chemical analysis.

Both the F_{2a1} and F_3 histones were found to contain significant amounts of methyllysine. The products of methylation in the F_{2a1} histones were ϵ -N-monomethyllysine and ϵ -N-dimethyllysine. The products of methylation in the F_3 histones were ϵ -N-monomethyllysine, ϵ -N-dimethyllysine and ϵ -N-trimethyllysine (Figures 17-18). No methylhistidine or methylarginine was found in any of the histone analyzed. Tables 2-6 show the distribution of the basic amino acids in the F_{2a1} histones in the various organs studied. The amounts of ϵ -N-monomethyllysine in all the organs except cerebrum were too low

TABLE 2

THE DISTRIBUTION OF BASIC AMINO ACIDS IN THE F_{2a1}
HISTONE FROM RAT CEREBELLUM

Basic Amino Acids	μmoles/mg	cpm/mg	³ H	cpm/μmoles
Lysine	0.90	8,650		9,600
ε-N-Monomethyllysine	Trace (0.007)*	70		--
ε-N-Dimethyllysine	0.061 (0.070)*	670		10,900
Histidine	0.19	0		--
Arginine	1.17	0		--

Forty-six newborns received two injections of 5.0 μcuries L-³H/lysine and 2.5 μcuries L-¹⁴C-methyl/methionine per gram of body weight at days 1 and 2 after birth. The animals were killed 13 days after the last injection. The cerebellums were removed and placed in cold 0.32 M sucrose. The nuclei were prepared and the F_{2a1} histone extracted from the nuclei as described under Materials and Methods. The histone was further purified by passage through Bio-Gel P-10 and its purity checked by polyacrylamide gel electrophoresis. Fractionation and quantitation of the basic amino acids were carried out as described under Materials and Methods. Radioactivity of the basic amino acids was determined by using a Packard Tri-carb scintillation counter.

* Calculated by dividing cpm/mg ³H in monomethyllysine or dimethyllysine by cpm/μmoles ³H in lysine.

TABLE 3

THE DISTRIBUTION OF BASIC AMINO ACIDS IN THE F_{2a1}
HISTONE FROM RAT CEREBRUM

Basic Amino Acids	μmoles/mg	cpm/mg ³ H	cpm/μmoles
Lysine	0.80	7,150	8,900
ε -N-Monomethyllysine	0.011 (0.011) *	103	9,400
ε -N-Dimethyllysine	0.071 (0.072) *	640	9,000
Histidine	0.17	0	--
Arginine	1.06	0	--

59

Forty-six newborns received two injections of 5.0 μcuries L-³H/lysine and 2.5 μcuries L-¹⁴C-methyl/methionine per gram of body weight at days 1 and 2 after birth. The animals were killed 13 days after the last injection. The cerebrums were removed and placed in cold 0.32 M sucrose. The nuclei were prepared and the F_{2a1} histone extracted from the nuclei as described under Materials and Methods. The histone was further purified by passage through Bio-Gel P-10 and its purity checked by polyacrylamide gel electrophoresis. Fractionation and quantitation of the best amino acids were carried out as described under Materials and Methods. Radioactivity of the basic amino acids was determined by using a Packard Tri-carb scintillation counter.

* Calculated by dividing cpm/mg ³H in monomethyllysine or dimethyllysine by cpm/μmoles ³H in lysine.

TABLE 4.

THE DISTRIBUTION OF BASIC AMINO ACIDS IN THE F_{2a1}
HISTONE FROM RAT LIVER

Basic Amino Acids	μmoles/mg	cpm/mg ³ H	cpm/μmoles
Lysine	0.86	7,600	8,800
ε -N-Monomethyllysine	Trace (0.005)*	45	--
ε -N-Dimethyllysine	0.066 (0.066)*	580	8,800
Histidine	0.20	0	--
Arginine	1.11	0	--

60

Forty-six newborns received two injections of 5.0 μcuries L- $\overline{^3\text{H}}$ lysine and 2.5 μcuries L- $\overline{^{14}\text{C}}$ -methylmethionine per gram of body weight at days 1 and 2 after birth. The animals were killed 13 days after the last injection. The livers were removed and placed in cold 0.32 M sucrose. The nuclei were prepared and the F_{2a1} histone extracted from the nuclei as described under Materials and Methods. The histone was further purified by passage through Bio-Gel P-10 and its purity checked by polyacrylamide gel electrophoresis. Fractionation and quantitation of the basic amino acids were carried out as described under Materials and Methods. Radioactivity of the basic amino acids was determined by using a Packard Tri-carb scintillation counter.

*Calculated by dividing cpm/mg $\overline{^3\text{H}}$ in monomethyllysine or dimethyllysine by cpm/μmoles $\overline{^3\text{H}}$ in lysine.

TABLE 5

THE DISTRIBUTION OF BASIC AMINO ACIDS IN THE F_{2a1}
HISTONE FROM RAT THYMUS

Basic Amino Acids	μmoles/mg	cpm/mg ³ H	cpm/μmoles
Lysine	0.88	12,700	14,400
ε-N-Monomethyllysine	Trace (0.004)*	60	--
ε-N-Dimethyllysine	0.061 (0.065)*	930	15,000
Histidine	0.154	0	--
Arginine	1.15	0	--

19

Forty-six newborns received two injections of 5.0 μcuries L- $\overline{^3\text{H}}$ lysine and 2.5 μcuries L- $\overline{^{14}\text{C}}$ -methyl/methionine per gram of body weight at days 1 and 2 after birth. The animals were killed 13 days after the last injection. The thymus were removed and placed in cold 0.32 M sucrose. The nuclei were prepared and the F_{2a1} histone extracted from the nuclei as described under Materials and Methods. The histone was further purified by passage through Bio-Gel P-10 and its purity checked by polyacrylamide gel electrophoresis. Fractionation and quantitation of the basic amino acids were carried out as described under Materials and Methods. Radioactivity of the basic amino acids was determined by using a Packard Tri-carb scintillation counter.

* Calculated by dividing cpm/mg $\overline{^3\text{H}}$ in monomethyllysine or dimethyllysine by cpm/μmoles $\overline{^3\text{H}}$ in lysine.

TABLE 6

THE DISTRIBUTION OF BASIC AMINO ACIDS IN THE F_{2a1}
HISTONE FROM RAT KIDNEY

Basic amino acids	μmoles/mg	cpm/mg	³ H	cpm/μmoles
Lysine	0.81	10,300		12,800
ε-N-Monomethyllysine	Trace (0.004)*	45		--
ε-N-Dimethyllysine	0.055 (0.058)*	740		13,400
Histidine	0.21	0		--
Arginine	1.11	0		--

Forty-six newborns received two injections of 5.0 μcuries L-³H-lysine and 2.5 μcuries L-¹⁴C-methyl-methionine per gram of body weight at days 1 and 2 after birth. The animals were killed 13 days after the last injection. The kidneys were removed and placed in cold 0.32 M sucrose. The nuclei were prepared and the F_{2a1} histone extracted from the nuclei as described under Materials and Methods. The histone was further purified by passage through Bio-Gel P-10 and its purity checked by polyacrylamide gel electrophoresis. Fractionation and quantitation of the basic amino acids were carried out as described under Materials and Methods. Radioactivity of the basic amino acids was determined by using a Packard Tri-carb scintillation counter.

*Calculated by dividing cpm/mg ³H in monomethyllysine or dimethyllysine by cpm/μmoles ³H in lysine.

TABLE 7

THE DISTRIBUTION OF ϵ -N-MONOMETHYLLYSINE AND ϵ -N-DIMETHYLLYSINE
IN THE F_{2a1} HISTONE FROM THE VARIOUS ORGANS OF THE RAT

Organ	Monomethyllysine $\mu\text{moles/mg}$	Dimethyllysine $\mu\text{moles/mg}$	Total Methyllysine	Moles/Mole Histone
Cerebellum	0.007	0.061	0.068	0.77
Cerebrum	0.011	0.071	0.082	0.93
Liver	0.005	0.066	0.071	0.80
Thymus	0.004	0.061	0.065	0.73
Kidney	0.004	0.055	0.059	0.67

to be chemically quantitated. The amounts were calculated by dividing the cpm/mg $\overline{[^3\text{H}]}$ in the monomethyllysine peak by the cpm/ $\mu\text{mole } \overline{[^3\text{H}]}$ in the lysine peak assuming the specific activity in both the amino acids was the same. Cerebrum had the highest amount of ϵ -N-monomethyllysine of all the organs studied. The value obtained by chemical analysis was the same as the calculated one (Table 3). The content of ϵ -N-dimethyllysine in the various organs was chemically quantitated. The values were in close agreement with the values obtained by dividing cpm/mg $\overline{[^3\text{H}]}$ in the dimethyllysine peak by cpm/ $\mu\text{mole } \overline{[^3\text{H}]}$ in the lysine peak (Tables 2-6). There was no significant difference in the content of dimethyllysine in the $\text{F}_{2\text{a1}}$ histone from the various organs studied; except in the kidney $\text{F}_{2\text{a1}}$ which appeared to be slightly lower in the content of dimethyllysine. Table 7 summarizes the total amount of methylated amino acid residues in the $\text{F}_{2\text{a1}}$ histone from the various organs studied. The organ that had the highest amount of methylated amino acids was cerebrum followed by liver, cerebellum, thymus and kidney.

DISCUSSIONS

The experiments reported here were designed to determine the distribution of methylated basic amino acids in the various histones from different organs of the rat, and also to determine the distribution of the different histones from organ to organ. The association of histones with DNA in the nucleus has prompted the study of the role of these basic proteins in the genetic processes of the cell. Among various hypothesis concerning the function of these proteins, the proposition advanced by Stedman and Stedman (31) that histones may interact with DNA in a specific manner, thereby influencing the phenotype of a cell, has stimulated a search for species and tissue specificity among these proteins. This search has yielded meager results. These proteins have been greatly conserved over the course of evolution and their exact biological function has not yet been delineated.

This investigation revealed that there was no significant difference in the distribution of the various histone components in the rat cerebellum, cerebrum, liver, thymus and kidney as detected by polyacrylamide gel electrophoresis. Bustin and Cole (32) reported the existence of microheterogeneity in the very lysine-rich histone (F_1). Very lysine-rich histones were extracted from chicken liver, calf thymus, and the thymus, mammary gland and liver of rabbit.

The elution profiles of these histones, obtained by chromatography on Amberlite IRC-50, revealed that the very lysine-rich histone complement varied from one animal species to another when a single kind of organ was considered, and even varied among different organs of a single species. Bustin and Cole (32) suggested that the very lysine-rich complement of any species consists of a moderately large number of molecular types. However, electrophoresis could not distinguish among the very lysine-rich histones of different tissues. Fambrough, Fujimura and Bonner (33) have also demonstrated variation in the quantitative distribution of several histone components in different pea organ. The very lysine-rich F_1 histone was found to show the most variation. Thus, it appears that the F_1 histone, at least in this respect, exhibit some form of microheterogeneity.

There did not appear to be enough difference in the distribution of methylated amino acid residues in the histones from the various organs of the rat to allow for tissue heterogeneity. In all the organs studied only the F_{2a1} and F_3 histones were found to be methylated. The products of methylation in the F_{2a1} histones were ϵ -N-monomethyllysine and ϵ -N-dimethyllysine, with a predominance of ϵ -N-dimethyllysine. The products of methylation in the F_3 histones were ϵ -N-monomethyllysine, ϵ -N-dimethyllysine and ϵ -N-trimethyllysine, with a predominance of ϵ -N-dimethyllysine and ϵ -N-trimethyllysine. The total ϵ -N-methyllysine content of the F_{2a1} histones varied from 0.67 moles/mole in the kidney to 0.93 moles/mole in the cerebrum. This variance in the methyllysine content is not appreciable, however, the values obtained were reproducible upon repeated experimentation.

The slightly higher methyllysine content in the cerebrum and liver may reflect the maturity of those cells in these particular organs. Allfrey et al (9), Duerre and Lee (10-11) have reported that methylation of the lysyl residues in the polypeptide chain is a relatively late event. Duerre and Lee (10-11) have also shown that methylation occurs after histones are bound to DNA. Consequently methylation of the histones in newly formed cells might be slightly less than in older, matured cells.

The significance of methylation is not understood. Methylation may have an effect on the interaction between histone and DNA. Since methylation of lysyl residues increases the hydrophobicity of these residues as well as their positive charge, methylation could result in an increased binding of histone to DNA, stabilizing the complexes which might contain information which is meant to be permanently suppressed.

In all the histones analyzed from these five organs, no methylarginine was detected. A few researchers (21-22) had reported the findings of methylarginine in rat brain, liver, thymus and kidney. Duerre and Lee (10-11) in their studies of brain histones, both in vivo and in vitro, reported that no methylarginine was detected in any of the histone components analyzed. However, analysis of microsomal protein in this laboratory showed the presence of methylarginine (34). One would expect to find methylarginine in histones if they were contaminated with microsomal protein.

The role that histones play in the biological function of the cell is still not yet understood. Studies done to date indicate that over the evolutionary process functional requirements would not permit the survival of an organism with much variation in the structure of these proteins. The mechanism by which the selection and regulation of genetic potential is accomplished in higher organisms is still largely unknown, and presents one of the most challenging problems in modern biology.

SUMMARY

Total histones were extracted from the cerebellum, cerebrum, liver, thymus and kidney of thirty 12 day old albino rats. Polyacrylamide gel electrophoresis of twenty-five μ g of histone from the various organs was carried out. The F_1 , F_3 , F_{2a2} plus F_{2b} and F_{2a1} components were separated into distinct bands and the relative per cent of the various bands on the gels were determined by scanning the gels through a densitometer. The relative distribution of the histone components from the various organs was compared. It was found that there was no significant difference in the distribution of the histone components from the organs studied.

The five histone components (F_1 , F_{2a2} , F_{2b} , F_3 and F_{2a1}) from the nuclei of the cerebellum, cerebrum, liver, thymus and kidney of forty-six 15 day old albino rats, which had previously received 10 μ curies of L- $[\text{3H}]$ lysine and 5 μ curies of L- $[\text{14C-methyl}]$ methionine, were extracted. The animals were sacrificed 14 days after the injection of the isotopic compounds. The various histone components from the various organs were further purified by gel filtration and their purity checked by polyacrylamide gel electrophoresis. The purified histone components were hydrolyzed in 6 N HCl in vacuo and the basic amino acids fractionated on Beckman P-35 resin. Quantitation of the basic amino acids were carried out with the aid of an automatic amino acid analyzer. It was found that only the

F_{2a1} and F_3 histones were methylated significantly. The products of methylation in the F_{2a1} histones were ϵ -N-monomethyllysine and ϵ -N-dimethyllysine, with a predominance of ϵ -N-dimethyllysine. The products of methylation in the F_3 histones were ϵ -N-monomethyllysine, ϵ -N-dimethyllysine and ϵ -N-trimethyllysine, with a predominance of ϵ -N-dimethyllysine and ϵ -N-trimethyllysine. No methyl arginine was detected in any of the histones analyzed. The amount of methyllysine in the F_{2a1} histones varied from 0.67 moles/mole in kidney to 0.93 moles/mole in cerebrum. Liver F_{2a1} had the second highest amount of methyllysine next to cerebrum. Pure F_3 histones were obtained by repeated gel filtration after which quantities available did not allow for complete analysis.

APPENDIX

PREPARATIONS OF REAGENT SOLUTIONS

Polyacrylamide Gel ElectrophoresisSolution A

60% Acrylamide and 0.4% N,N'-bisacrylamide in water

Solution B

43.2% Glacial acetic acid and 4% TEMED (N,N,N',N'-tetramethyl-ethylenediamine) in water

Solution C

0.2% $(\text{NH}_4)_2\text{S}_2\text{O}_8$ in 10 M Urea.

Tracking dye

0.1% Safranin in 7% Acetic acid and 5% sucrose.

Staining solution

1.0 % Buffalo black in 20% Ethanol and 7% Acetic acid.

Bray's Counting Solution

100% Ethanol	200 ml
Toluene	800 ml
PO 2,5 Diphenyloxazole	6 g/liter
POPOP 1,4-bis- $\sqrt{2}$ -(5-phenyloxazolyl) $\sqrt{2}$ -Benzene ..	100 mg/liter

Determination of Protein (Lowry)Reagent A

2.0% Na_2CO_3 in 0.1 N NaOH

Reagent B

0.5% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in water

Reagent C

1.0% Na or K tartrate in water

Reagent D

Reagent A	50 ml
Reagent B	0.5 ml
Reagent C	0.5 ml

LITERATURE CITED

1. DeLange, R. J., Fambrough, D. M., Smith, E. L., and Bonner, J. (1969). J. Biol. Chem. 244, 5669-5679.
2. Bonner, J., and Garrard, W. T. (1974). Life Sciences 14, 209-221.
3. Allfrey, V. G. (1971). in Histones and Nucleohistones (Philips D. M. P. ed) pp. 241-294, Plenum Press, New York.
4. DeLange, R. J., Smith, E. L., Fambrough, D. M., and Bonner, J. (1968). Proc. Nat. Acad. Sci. U. S. 61, 1145-1147.
5. Ambler, R. C., and Rees, M. W. (1959). Nature 184, 56-57.
6. Kerridge, D. (1963). J. Gen. Microbiol. 31, Proceedings, XXIV-XXVII.
7. Murray, K. (1964). Biochem. 3, 10-14.
8. Allfrey, V. G., Faulker, R., and Mirsky, A. E. (1964). Proc. Nat. Acad. Sci. U. S. 51, 786-794.
9. Tidwell, T., Allfrey, V. G., and Mirsky, A. E. (1968). J. Biol. Chem. 243, 707-715.
10. Duerre, J. A., and Lee, C. T. (1974). J. Neurochem. 23, 541-547.
11. Duerre, J. A., and Lee, C. T. (1974). Nature 251, 240-241.
12. Kim, S., and Paik, W. K. (1965). J. Biol. Chem. 240, 4629-4634.
13. Alvin, M. K., and Dalia, S. (1960). Biochim. Biophys. Acta 190, 527-537.
14. Paik, W. K., and Kim, S. (1970). J. Biol. Chem. 245, 6010-6015.
15. Paik, W. K., Lee, H. W., and Morris, H. P. (1972). Cancer Res. 32, 37-46.
16. Paik, W. K., and Kim, S. (1973). Biochem. Biophys. Res. Commun. 51, 781-788.
17. Paik, W. K., and Kim, S. (1967). Biochem. Biophys. Res. Commun. 27, 479-483.
18. Paik, W. K., and Kim, S. (1967). Biochem. Biophys. Res. Commun. 29, 14-20.

19. Hempel, K., Lange, H. W. and Birkhofer, L. (1968).
Z. Physiol. Chem. 349, 603-703.
20. DeLange, R. J., Hooper, J. A., and Smith, E. L. (1973).
J. Biol. Chem. 248, 3261-3274.
21. Byroet, P. (1971). Biochim. Biophys. Acta 238, 375-376.
22. Paik, W. K., and Kim, S. (1969). J. Neurochem. 16, 1257-1261.
23. Shepherd, G. R., Hardin, J. M., and Noland, B. J. (1971).
Arch. Biochem. Biophys. 143, 1-5.
24. Borun, T. W., Pearson, D., and Paik, W. K. (1972). J. Biol.
Chem. 247, 4288-4298.
25. Desai, L. S., and Foley, G. E. (1970). Biochem. J. 119, 165-170.
26. Johns, W. E. (1964). Biochem. J. 92, 55-59.
27. Duerre, J. A., and Gaitonde, M. K. (1971). J. Neurochem. 18,
1921-1929.
28. Panyim, S., and Chalkley, R. (1969). Arch. Biochem. Biophys.
130, 337-346.
29. Reisfeld, R. A., Lewis, U. J., and Williams, D. E. (1962).
Nature 195, 281-283.
30. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall,
R. J. (1951). J. Biol. Chem. 193, 265-275.
31. Stedman, E., and Stedman, E. (1950). Nature 166, 780-781.
32. Bustin, M., and Cole, R. D. (1968). J. Biol. Chem. 243,
4500-4505.
33. Fambrough, D. M., Fujmura, F., and Bonner, J. (1968). Biochem.
7, 575-584.
34. Duerre, J. A., Unpublished data.